INDIVIDUAL GROWTH VARIABILITY IN FARMED ATLANTIC SALMON (*SALMO SALAR L.*): THE ROLE OF SOCIAL INTERACTIONS

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Two Atlantic salmon competing for feed in an experimental tank

Declaration

I declare that the work described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It is entirely of my own composition and has not, in whole, or in part, been submitted for any other degree.

K Fiona Cubitt

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ABSTRACT

This thesis examines the development and persistence of size variation in Atlantic salmon (*Salmo salar*). A series of linked studies characterised individuals displaying marked differences in growth trajectories. These studies investigated size variation from a behavioural perspective and provided information that may be applied to the commercial production of Atlantic salmon.

Chapter 2. Tracked the development of size variation in semi commercial conditions. A six month long study allowed the identification of sub groups of fish displaying distinct growth trajectories. These sub groups were apparent as lower, upper and main modes in the weight distribution of each population.

Chapter 3. Investigated behavioural differences in three sub-groups of varying sizes. This revealed that lower mode fish were behaviourally distinct from small fish in the main mode and from large fish (the largest fish in the main mode and the upper mode fish combined). Lower mode fish fed at different times of the day from large fish and occupied different areas of the tank. In addition, the proportion of large fish feeding was greater when competition for food was increased, and lower when competition for food was decreased. Lower mode fish displayed the opposite pattern; a greater proportion fed when competition for food was decreased. These results suggest that lower mode fish were excluded from feeding at the same times as large fish either because of direct competition or due to social interactions.

Chapter 4. Demonstrated a biochemical technique to recreate previous social status. This technique provided strong evidence that social hierarchies can occur in relatively large groups at high densities. The results from this study suggest the occurrence of a long-term linear hierarchy, with consistently large fish remaining dominant and a small group of profoundly subordinate individuals. However, it is likely that there was also a continually changing group of fish that became dominant for short periods.

Chapter 5. Investigated the capacity of non-growing fish to grow.

Non-growing fish from the long-term study on the development of size variation (Chapter 2) provided the opportunity to relate the effects of season and of larger individuals on the performance of non-growing fish. These previously non-growing individuals grew after the summer solstice, but those in the absence of larger fish exhibited a faster and greater recovery than those in the presence of larger fish.

Chapter 6. Investigated the concept of intrinsic competitive ability.

The occurrence of differential growth rates in initially tightly graded populations (Chapter 2) suggested that particular individuals were predisposed to perform well. Thus, the growth of good performers were compared with size-matched poor performers. This revealed that previous performance (which may be confounded by previous winning experience) provided an initial, short term growth advantage.

Chapter 7. General discussion

Brings together the findings and concepts of the previous chapters and discusses the implications that these findings have for commercial farming of Atlantic salmon.

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CHAPTER 1. GENERAL INTRODUCTION

1. 1 THE ECONOMICS OF SALMONID AQUACULTURE

Aquaculture originated in eastern Asia four thousand years ago, and is now extremely widespread. One billion people world-wide rely on fish as their primary source of animal protein and the Food and Agriculture Organisation of the United Nations (FAO, 2000) estimates that by 2030, over half of the fish consumed will be supplied by aquaculture. Many fish species have a high protein content and fish muscle contains fatty acids that have been shown to benefit human health (Monahan, 1993). In addition, aquaculture often provides jobs in remote areas of high unemployment and has been growing six times faster in developing countries than developed countries (Tidwell & Allan, 2001). Aquaculture produced 38 million tonnes of fish worth US$ 22.5 billion in 1998 (FAO, 2000).

Salmonids are one of the most common groups of farmed fish. Pacific salmon, charrs and trouts mainly farmed for stock enhancement, whereas rainbow trout (see Appendix I for scientific names) and Atlantic salmon are also farmed for food production (Jobling, 1993). The Atlantic salmon is the most widely cultured of the salmonids; 800,000 tonnes were produced worldwide in 1999 (FAO, 2000). Atlantic salmon farming originated in Norway in 1965 as an alternative to trout farming, but the current geographical range of salmon farming is much larger than the natural range of the species. In the wild, salmon exist between 40°N and 70°N in the North Atlantic Ocean and the rivers of North America, Scandinavia and Europe (MacCrimmon & Gots, 1979). However, the range of farmed Atlantic salmon includes the Southern Hemisphere and the Pacific Ocean (Heen *et al.*, 1993).

1. 2 DOMESTICATION AND BREEDING PROGRAMS

Atlantic salmon have been farmed for a relatively short period of time, but during this time they have become different from their natural ancestors. It has even been suggested that farmed Atlantic salmon should be classified as an independent species from wild Atlantic salmon (Gross, 1998). Many of the factors that shape the phenotype and ultimately, through natural selection, the genotype of an animal, are absent for farmed fish. The aquaculture environment is homogeneous, there are few predators and there is a reliable food supply. As a result, farmed fish often lack behavioural traits shown by their wild ancestors; e.g. hatchery brown trout show reduced antipredator responses (Johnsson *et al.* 1996) and medaka show reduced aggression following domestication (Ruzzante and Doyle 1993).

In addition, breeding programs select for favourable characteristics. Fast growth is one such characteristic, and has been repeatedly and successfully selected for. Growth increases of 3.6% per year have been reported from selection programs (Gjedrem, 1983). As a result of artificial selection, environmental manipulation and refinement of production techniques, Atlantic salmon can be grown to production size in at least half of the time it would take in the natural environment.

Despite a growing body of research, the extent and the effects of salmonid domestication are unclear and both are likely to be different in groups subjected to different selective pressures (e.g. Ruzzante, 1994). Research into the domestication of Atlantic salmon can provide widely applicable insights on the process of domestication in general. Furthermore, techniques and knowledge gained from this research may be applicable to both current and future domesticated aquaculture and agriculture species.

1. 3 LIFE HISTORY OF WILD ATLANTIC SALMON

In nature, mature Atlantic salmon females spawn on gravel stream-beds between late autumn and early winter. In early spring the young alevins hatch from the eggs and remain in the gravel bed until they have absorbed their yolk sacs (Scott & Crossman, 1973). Once the yolk sacs are absorbed they emerge from the gravel as fry around the middle of spring (Dill, 1977). As they begin to feed they compete for access to the best territories on the stream-bed (Kalleberg, 1958). The fish are known as parr from this time until they migrate to sea. The time of migration and therefore smolting, is determined by growth rates between April and July; those with higher growth rates migrate during their first year, whereas those with slower growth rates are more likely to delay migration until at least the following year (Thorpe, 1977). Individuals can remain in freshwater for up to six years before migrating, but two to three years are more likely. However, some individuals mature in freshwater and do not go to sea. Once the fish have migrated to the sea they feed and grow intensively and return to their native rivers after two years. Recent evidence suggests that migrating Norwegian Atlantic salmon are found between Norway and the Faroe Islands and Iceland (Holm *et al.*, 2000). The life of Atlantic salmon once they have gone to sea is poorly researched in comparison to their juvenile, freshwater phases.

1. 4 MANAGEMENT OF ATLANTIC SALMON LIFE HISTORY IN AQUACULTURE

Salmonid aquaculture in the present day provides a continuous supply of fish all year round; a combination of light and temperature manipulation results in many harvests each year. The basic production procedure is similar for all strains irrespective of the degree of temperature and light manipulations to which they are exposed. Initially, eggs are brought into the hatchery (usually in November or December) where they are exposed to tightly controlled conditions and a high level of hygiene. In the wild, the

survival rate from egg to maturity is 0.12%, but in commercial conditions this is increased to 60 – 80% (Willoughby, 1999). In the hatchery the eggs are typically incubated at low temperatures (4 - 8°C), exposed to low light levels and highly filtered freshwater. As mentioned in the life history of natural salmonids, alevins hatch from the eggs and initially feed on their yolk sacs, water temperature may be raised at this point to aid development. First feeding commences four to six weeks after hatching and fish are termed fry from this point. The fry grow rapidly, increasing their bodyweight by five to seven percent each day. As the fry grow there is a high degree of size variation. This allows the fish to be graded into groups of slightly different sizes that can be harvested at slightly different times. The fish are then grown in cages or tanks in freshwater until they undergo smoltification allowing them to osmoregulate in seawater.

Under traditional methods of Atlantic salmon production, juveniles are separated into two groups; those that will undergo smoltification in their first spring (S1 fish) and those that will undergo smoltification the following year (S2 fish). However, advances in photoperiod manipulation, temperature control and feed nutrition have lead to the introduction of S½ and S1½ smolts. When smolts are judged to be ready for transfer to seawater (the timing of which is usually ascertained by a 24-hour seawater salinity tolerance test of a small sample of individuals), they are transferred to sea cages where they remain until harvest.

The size of sea cages and hence the number of individuals within them varies from farm to farm and country to country. Galvanised steel cages with 15m x 15m x 15m deep nets are one of the most commonly found type of cages. These cages were popular in the 1980s, and still are. However, there is currently a trend for cages of an increasing size, with 100m diameter circular cages being popular in northern Norway. As fish have been repeatedly observed to shoal and swim in a circular motion, circular cages may enable fish to make the best use of space (Juell & Westerberg, 1993).

However, in all cages, commercial densities are usually between 12 – 15kg·m^{-3} (A. Melstveit, pers com). The majority of salmonid sea cages are found along the coastline in fjords and lochs. Until recently, there were very few studies on the behaviour and social interactions of Atlantic salmon in sea cages. However, the initial documentation of social interactions (e.g. Thorpe *et al.*, 1990; Juell, 1995; Kadri *et al.*, 1996a) has prompted the need for further studies.

In 1996, the Farm Animal Welfare Committee published a set of guidelines on the wellbeing of farmed fish (FAWC, 1996). Until this time there was relatively little concern for the welfare of commercially farmed fish. Furthermore, in contrast to other areas of intensive farming e.g. poultry and pigs, there are relatively few regulations or guidelines on the welfare of farmed fish. However, the UK government has recently funded projects investigating, amongst other things, humane slaughtering methods and the effect of high stocking densities on the welfare of farmed fish. In addition, there is an increasing public demand for high standards of animal rearing.

1. 5 ENVIRONMENTAL ISSUES

Due to the large number of individuals held at high densities in sea cages, there is a high degree of waste. The greatest proportion of this waste is uneaten food (Beveridge, 1996), therefore causing a high degree of sedimentation. The degree of sedimentation decreases with increasing distance between the seabed and the cage, and with increasing current flow. Initially, the increase in nutrients will increase the oxygen demands of the sediments. However, where there is a high degree of sedimentation this can result in anoxic areas below cages, where the structure of the natural community is completely changed. Waste can adversely affect the populations within the cages; deoxygenation of water can occur as a result of increased sediment oxygen demands (Lumb, 1989), and methane and hydrogen sulphide have been

implicated in outbreaks of gill disease in Norwegian fish farms (Black *et al.*, 1994). Waste from sea cages can be collected e.g. the Lift-up system developed by Refa A/S (Juell *et al.*, 1993), but the most beneficial solution, economically and in terms of the environment is to reduce the amount of waste (Beveridge, 1996).

1. 6 VARIABILITY IN GROWTH IN AQUACULTURE

There have been many advances in the technology and selective breeding of salmonids, but pressures of competition in the market, issues of welfare for the fish and rising feed costs ensure that research into improved growth rates and product quality continue. Many of the changes that are made as salmonid aquaculture progresses raise new questions. For example, artificial selection may cause unforeseen problems; faster growing fish can be more aggressive or competitive, so selection for faster growth may enhance such behaviours rather than maximising growth efficiency (Swain & Riddell, 1990; Johnsson *et al.*, 1996).

As feed costs make up 50-60% of production costs (Sveier & Lied, 1998), there is a large body of research investigating this area of aquaculture. The nutritional composition of feed and methods of feeding have been extensively refined resulting in feed conversion rates of 2.3 in 1980 being improved to as good as 1.1 in recent years (Willoughby, 1999). Although there are improvements that can still be made to the composition of feed, research in this area does not provide the high returns that it did previously. As a result of this, there is an increasing body of research on the social interactions and feeding behaviour of farmed fish (V Crampton, pers com).

One major problem faced by aquaculturists is that of variation in fish size. Consumer demand for a high quality, uniform product means that small, poor quality fish result in lost revenue for the farmer, both in terms of market price and feed investiture during rearing. The occurrence of larger fish also suggests that smaller fish are not

expressing their full growth potential. In addition, the presence of small fish in conditions of adequate food supply raises questions about the welfare of these individuals. A detailed study of the development and nature of such variation can provide indications of the causes of such variation.

Differences in growth, and the resulting size variation are often linked to competition for food as low levels of feed result in increased size variation (Davis & Olla, 1987; McCarthy *et al.*, 1992a). Increasing feed levels does reduce the degree of size variation, but only to a certain degree. In addition, this is not an economically viable option in commercial conditions as feed waste increases more than the resultant decrease in size variation. Furthermore, waste feed is detrimental to the environment (see above). The persistence of size variation in populations exposed to high ration levels suggests that social interactions depress the growth of some individuals (Jobling, 1995). Thus, it is important to establish the behavioural differences between individuals showing different patterns of growth.

1. 7 COMPETITION AND DOMINANCE IN NATURE

In nature, the ability of an individual to consistently obtain access to resources, amongst other things, can determine the growth of that individual. Irregularities in growth within species are common and are often attributed to differences in competitive ability. Competition increases when resources are in short supply or are monopolisable (e.g. Magnuson, 1962; Thorpe *et al.*, 1990; Kadri *et al.*, 1996a).

Competition is often structured in the form of dominant – subordinate relationships, where dominant individuals gain preferential access to resources. Dominance is initially established by repeated fighting and / or threats, but once the dominance – subordinate relationship is established there is often little evidence of aggression

(Wilson, 1975; O'Connor *et al.*, 1999). Perhaps the simplest form of a dominance relationship consists of one individual dominating all other individuals. This can be found in the social insects, e.g. ants, bees and wasps, and in a small number of social rodent species (Jarvis, 1981). Alternatively, hierarchies of dominance may exist with triangular or circular elements. However, such hierarchies often stabilise into linear hierarchies (e.g. Murchison, 1935; Tordoff, 1954), where each individual dominates those below it, and in turn is dominated by all individuals above it in the hierarchy. Dominance hierarchies are widespread in the animal kingdom and are found in mammals (e.g. rabbits, Albonnetti *et al.*, 1988; African dogs, Creel, 1997), birds (e.g. Willow tits, Ekman & Askenmo, 1984; greylag geese; Kotrschal *et al.*, 1998) and fish (e.g. coral reef damselfish, Booth,1995; green swordtail fish, Beaugrand & Cotnoir, 1996). Dominance hierarchies also occur in domesticated species (e.g. domestic fowl, Craig and Guhl, 1969; and pigs, Mendl *et al.*, 1992; goats, Barroso *et al.*, 2000).

Dominant individuals gain preferential access to resources, for example mating opportunities (e.g. stumptail macaque, Estep *et al.*, 1988; mountain goats, Cote & Festa-Bianchet, 2001) and feeding sites (e.g. yellow-eyed juncos, Caraco, 1979; female red deer, Clutton-Brock *et al.*, 1984). Dominant individuals also tend to be the largest in the hierarchy (Huntingford & Turner, 1987), although this is not always the case (e.g. male tilpaia, Neat *et al.*, 1998).

Similarly, in juvenile salmonids, dominant individuals may often gain access to the best territories or areas of the stream (Fausch 1984; Metcalfe 1986) and also tend to be the largest in the group (Wankowski & Thorpe 1979; Abbott *et al.*, 1985). However in salmonids, large size appears to be a consequence, rather than a cause of dominance status (Huntingford *et al.*, 1990).

In agreement with this, Koebele (1985) found that the occurrence of a dominance hierarchy resulted in disproportional food acquisition. This in turn was the main cause

of size differences in juvenile cichlids. However, Abbot and Dill (1989) attributed growth variation in subordinate and dominant juvenile steelhead trout fed on the same rations to effects of social stress. In order to ascertain whether size differences in farmed Atlantic salmon are the result of dominance hierarchies, it is necessary to relate the growth of known individuals to their behaviour in commercial conditions. However, behavioural observations are restricted by difficulties in monitoring behaviour under water and by large group sizes at high densities (see below). Therefore, the performance of individuals that appear to have undergone growth suppression could be monitored *post hoc* in the absence of larger fish to provide information on the influence of these large fish.

1. 8 STUDYING FISH BEHAVIOUR AT HIGH DENSITIES

The difficulties faced in studying the behaviour of large groups of land animals have been overcome by the use of satellite and radio tracking, (e.g. in elephants, Thouless 1996). However, the inability to observe the subject is perhaps the greatest hindrance in studying fish behaviour in aquaculture conditions. The behaviour of other sea animals can be monitored by satellite telemetry (e.g. seals, Davis *et al*,. 2000; turtles, Hays *et al.*, 2001). However, a combination of the relatively small size and the high densities of commercially farmed salmonids creates restrictions on the observation of these fish. A number of techniques have been developed to overcome this. Video cameras have been successfully used to monitor general swimming and feeding patterns of fish in sea cages (Philips, 1985; Kadri *et al.*, 1991). Furthermore, Petrell *et al.* (1997) demonstrated that swimming speeds could also be calculated when two cameras were used (see also Sheih & Petrell, 1998). Feeding behaviour can also be measured indirectly, by using data from feeding systems that adjust the level of feed delivery to the amount of waste feed (e.g. Blyth *et al.*, 1993). Demand feeding systems, where the fish bite or push a trigger to release food have been used to study feeding rhythms and food levels of consumption (Alanärä, 1992; Fast *et al.*, 1997). On

a smaller scale, the addition of PIT tags to a demand feeding system allowed information to be gathered on individual feeding patterns (Brännäs & Alanärä, 1993), but PIT tags are very difficult to use in seawater. Ultrasonic techniques for tracking fish, previously used in fisheries research, have been tested in sea cages (Juell and Westerberg, 1993; Bégout-Anras, 1995). These systems can give 3-dimensional co-ordinates for individual fish over an extended period of time. However, they involve the use of relatively large and expensive tags and can only monitor a limited number of individuals.

1. 9 AIMS AND OBJECTIVES

This study aims to investigate the development and nature of size variation of Atlantic salmon smolts in semi-commercial conditions, from a behavioural perspective. In particular, the following questions will be addressed:

- Is it possible to identify subsets of individuals following different growth trajectories?
- Do groups of fish following markedly different growth trajectories differ behaviourally?
- Do individuals of different performance have different social histories?
- Do slow growing individuals have the ability to grow fast in favourable conditions?
- Do individuals have intrinsic differences in competitive ability?

In order to answer these questions, a series of linked experiments were carried out in semi-commercial conditions; where information could be gathered with a view to future application in commercial Atlantic salmon production. Initially, the development of size variation was investigated in tightly graded populations. The growth of individuals was tracked and the effect of feeding intensity on the resultant size variation was monitored (Chapter 2). This study provided fish of a range of sizes, and hence performance, on which the mechanisms behind differential growth could be further examined. The

relative timing and organisation of the experiments conducted for this thesis are summarised in Figure 1.1

The following chapters provide information on subsets of individuals of differing size and therefore different performance ability. These individuals were provided by the study described in Chapter 2. Video observations of the spatial distribution and feeding behaviour of three size-classes of fish were collected and analysed in Chapter 3. However, as information on the behaviour of individuals is difficult to obtain from large, high-density groups, a technique that reconstructed individual social history was demonstrated in Chapter 4. During the initial development of size variation, a small proportion of individuals, from all feeding intensities, did not grow. Therefore, the relative effects of larger fish, and of season, on the growth of these individuals was monitored under conditions of plentiful food supply (Chapter 5). Finally, predictors of growth were examined in terms of previous performance and nutritional status (Chapter 6). The findings of these studies were then discussed in relation to relevant literature in Chapter 7.

The studies described in this thesis are written as discrete experiments. Therefore, the issue of size variation is introduced in each chapter with respect to the particular questions being addressed. This resulted in the repetition of information from the main introduction (Chapter 1) throughout the thesis.

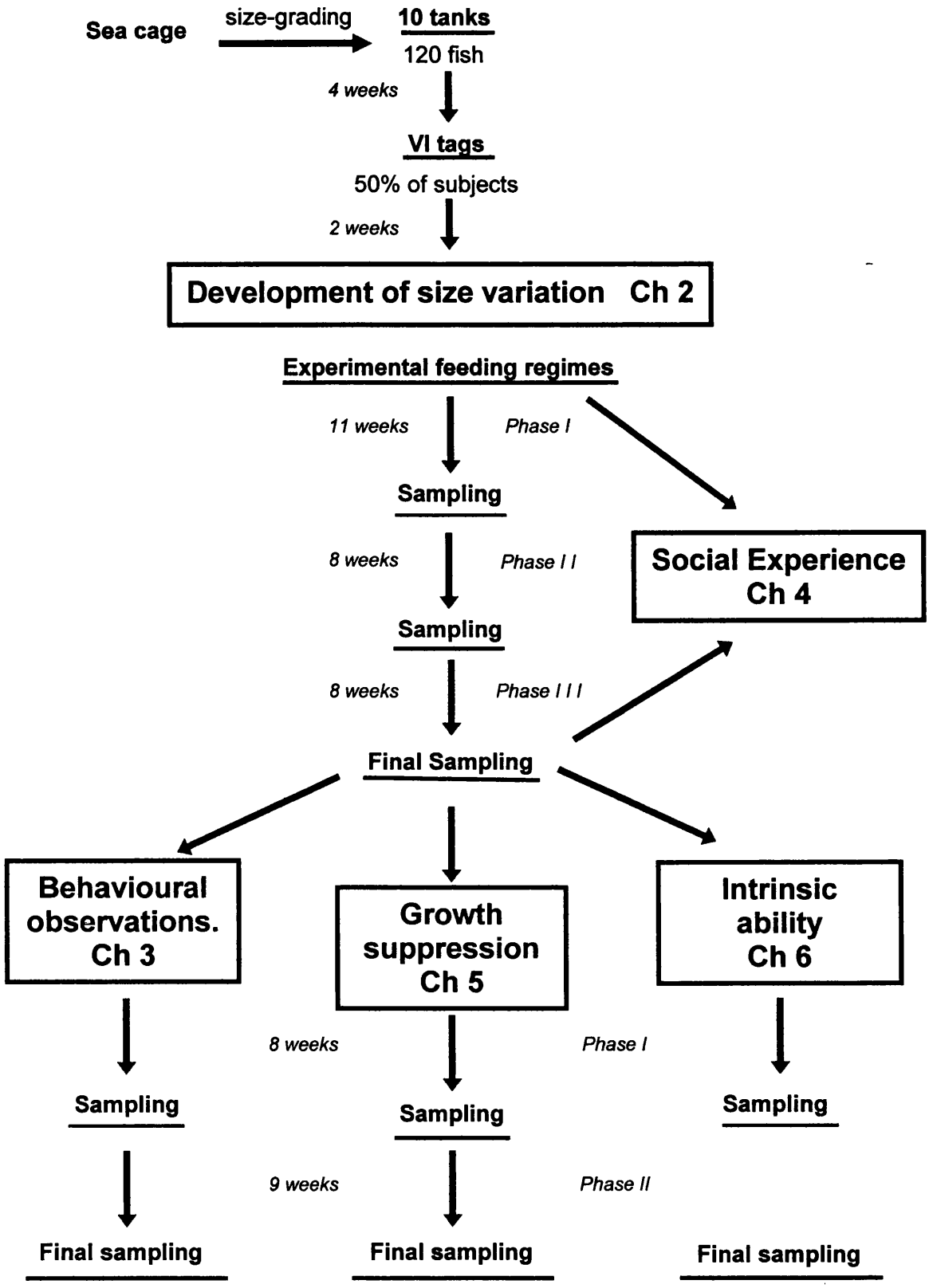


Figure 1.1 Summary of the experiments that make up this thesis.

CHAPTER 2. THE DEVELOPMENT OF SIZE VARIATION IN GROUPS OF ATLANTIC SALMON.

2. 1 INTRODUCTION

In aquaculture, individuals are reared in controlled conditions very different from those in nature; food is relatively abundant, the substrate is homogenous and the pressures of predation and disease are greatly reduced, or absent. However, despite this, farmed fish cultured in tanks and sea cages grow heterogeneously (Storebakken & Austreng, 1987a; Johansen & Jobling, 1998; Irwin *et al.*, 2002). The resultant size variation is problematic for farmers; small fish that are in poor condition cannot be sold and time and money will have been invested in these individuals to get them to this stage. In addition, the occurrence of particularly large individuals suggests that there are many fish that are not fulfilling their growth potential.

The processes that cause growth variation in aquaculture are unclear. However, biologists have investigated the mechanisms behind growth variation in natural populations (e.g. Bailey, 1980; Koebele, 1985; Metcalfe *et al.*, 1992). The information gained from these studies may help to elucidate the causes of size variation in aquaculture. Additionally, knowledge gained from the examination of size differentiation in aquaculture may be applicable to other large groups of animals both commercially (e.g. free-range chickens) and in the natural environment (e.g. deer).

In aquaculture, growth variation increases when groups of salmonids are exposed to low ration levels (Storebakken & Austreng, 1987b; Damsgard *et al.*, 1997). However, as feed costs make up 40 - 60% of total production costs (Juell *et al.*, 1994; Sveier &

Lied, 1998), and waste feed contributes to pollution, farmers cannot feed to excess. In addition, there are also indications that size variation occurs, but to a smaller extent, when feed availability is high (Koebele, 1985; Abbott & Dill, 1989; McCarthy *et al.*, 1992b).

In wild salmonids, size variation is often attributed to interactions between individuals. Large individuals are likely to be dominant, whereas smaller individuals are likely to be subordinate (Jenkins, 1969; Wankowski & Thorpe, 1979). Dominant individuals obtain more food and grow faster (Fausch, 1984; Metcalfe, 1986). These differences in feed intake have been reported to be the main cause behind variation in growth rates (Koebele, 1985; Jobling & Wandsvik, 1983; McCarthy *et al.*, 1992b). In larger groups, fish with the highest feeding ranks also grow fastest. This was demonstrated both by Carter *et al.* (1996) in groups of 20 flounder and by Irwin *et al.* (2002) in groups of 30 turbot. Furthermore, disproportionate distribution of food has been observed amongst Atlantic salmon in sea cages (Thorpe *et al.*, 1990). Therefore, similar mechanisms to those that mediate food distribution in small groups may also occur in large groups.

In the current study, the development of size variation was monitored at an individual and at a group level and distinct patterns of growth identified. The influence of feeding regime and feed intake on these growth patterns was elucidated.

2. 1. 1 Aims

To examine the development of size differences in initially graded populations of Atlantic salmon. In particular:

- to identify major differences in growth trajectory,
- to characterise fish following different growth trajectories,
- to examine the effects of ration on these processes.

2. 2 METHODS

In the present study, 10 populations of 120 adult Atlantic salmon were held at commercial densities and reared on a range of feeding rations; from maintenance to overfeeding. Half of the subjects were tagged, allowing these individuals to be tracked throughout the study. Data on fish weight, length, feed intake and minor injuries were collected at three points during the study, splitting the study into three phases.

2. 2. 1 Experimental housing

The study took place in circular, 3m diameter x 2m deep tanks supplied with seawater pumped from a depth of 30m, providing water with a median temperature of 8.4°C and a range of 6.4°C to 10.8°C (Figure 2.1). Water entered through a vertical pipe at the side of the tank at 210 $l.min^{-1}$ and exited from the centre of the tank base, giving a circular flow. The oxygen concentration in the outlet was maintained between 75% and 80% saturation for the duration of the trial; in sea cages the concentration is around 90% saturation (L. Oma, pers com). The tanks were illuminated lights for 12 hours each day, but small circular windows (30cm diameter) in the wall of each tank, 1m from the tank base, may have provided the subjects with ambient photoperiod cues. Figure 2.1 illustrates the number of hours between dawn and dusk during the study. This measure is longer than the actual number of daylight hours, but gives a relative measure for the change in daylength throughout the study. During the first phase of the study (a duration of 13 weeks), tanks fed on commercial feeding regimes (see below) were fitted with waste feed collectors to allow detailed monitoring of actual feed consumption. The feed collectors were removed at the end of phase I, as there was a significant increase in the incidence of skin injuries in these tanks. (chi-square test of association: $\chi^2 = 65.198$, d.f. = 1, $P < 0.001$). 12% of the fish in tanks with feed collectors sustained skin injuries, whereas only 1% of the fish in tanks without feed collectors were affected by skin injuries.

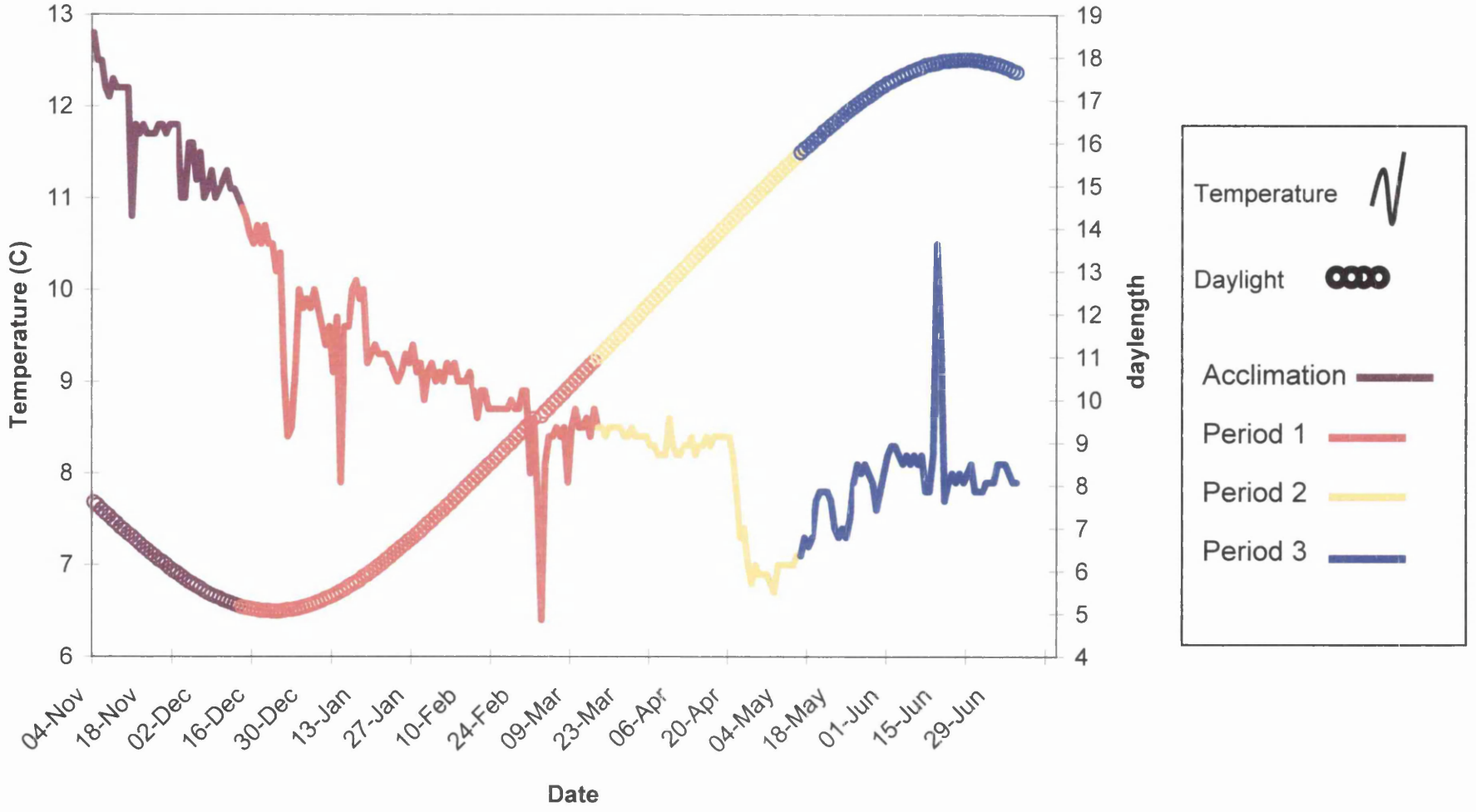


Figure 2.1 Temperature and daylength for each time period. during the study.

2. 2. 2 Subjects

In the week beginning the 1st of November 1999, 1200 sibling fish, with a mean weight of 1.34kg ± 3%, were transferred from a 15m x 15m x 15m deep cage into 10 experimental tanks, over 3 days. Each tank contained a population of 120 fish, giving a density of 15.16kg.m^{-3} (commercial densities for Atlantic salmon are usually in the range of 12-15kg.m^{-3}). The subjects underwent a four-week acclimation period, during which all populations were fed 0.5% body weight per day. In the week beginning the 13th of December, 60 individuals were removed from each tank, weighed, measured and fitted with VI tags (Northwest Marine Technology Ltd., Shaw Island, WA.) implanted in the post-ocular eyelid tissue (Niva 1995).

2. 2. 3 Feeding regime

The experimental feeding regimes began in January 2000, following a further two weeks of acclimation to enable the tagged fish to recover. The populations of fish were fed on a range of rations, from maintenance to excess, providing a range of competitive intensities. Commercial feed was used throughout the study (EWOS dynamic red, size L) and was delivered via a pipe that spanned the width of the tank. Feed was delivered from 07:00 until 19:00 at 7.5 minute intervals in all feeding regimes, with lower regimes having shorter periods of feed delivery. The feeding regimes were derived from a value used in commercial conditions of 0.5% body weight per day (bwpd); three tanks were fed at this level. Two tanks were fed on a maintenance ration of 0.15%bwpd, (30% of the commercial ration) and three tanks were fed on levels between maintenance and commercial rations: 0.25%bwpd, 0.35%bwpd and 0.45%bwpd, providing 50%, 70% and 90% of the commercial ration respectively. The remaining two tanks were fed to excess on 0.6%bwpd, 120% of the commercial ration. In all tanks, the amount of feed delivered was increased by 0.5% each day. Throughout this chapter the feeding regimes are referred to as a percentage of the commercial ration, for example a level of 0.5%bwpd is referred to as 100%. For the purpose of some

analyses, feeding regimes from 30% to 70% were termed 'low' and feeding regimes from 90%, to 120% were termed 'high'.

2. 2. 4 Analysis of effects of feeding intensity

The feeding regimes chosen for the study were on a ratio scale, facilitating regression analysis. The lowest and highest feeding regimes were replicated to increase the power of the regression. Additional information on the effects of under feeding, 'normal' feeding and over feeding from a commercial perspective was provided by the 90%, 100% and 120% feeding regimes respectively. Unfortunately, due to a technical problem, one tank on the 100% feeding regime suffered a sudden high mortality during the final phase (phase III). Consequently, it was removed from all subsequent analyses.

2. 2. 5 Sampling

The subjects were sampled three times. Sampling was carried out over four days during the weeks beginning the 13th of March, the 8th of May and the 3rd of July; 13, 21 and 29 weeks after tagging, respectively. The period between 0 and 13 weeks was termed phase I, phase II ran from 13 to 21 weeks and the remaining 8 weeks were termed phase III. During sampling, individuals were anaesthetised (Aqui-S, New Zealand), weighed, measured and evidence of injury was recorded (see below). Tagged individuals were identified in all tanks but in replicated tanks (those fed 30%, 100% and 120%), tagged fish were also x-rayed in order to measure feed intake.

2. 2. 6 Estimating feed intake

Prior to sampling, commercial feed in the replicated tanks (those on 30%, 100% and 120% feeding regimes) was replaced with feed labelled with X-ray opaque glass beads (Ballotini grade 8.5: 400-450μm diameter, Jencons Ltd., U.K.). The labelled feed was produced by adding Ballotini to a sample of the experimental feed. Homogeneity of the Ballotini within the mixture was ensured by X-raying the mixture before pelleting.

Ballotini-labelled feed was delivered under experimental feeding regimes for 4 hours during samplings at the end of phase I and phase II. Analysis of the X-ray plates (see below), revealed that a large number of fish had no food in their stomachs (72% at the end of phase I, 57% at the end of phase II). Feeding was then increased to 6 hours during the final sampling, at the end of phase III and this resulted in 40% of fish without food in their stomachs. X-raying commenced one hour after feeding finished to avoid regurgitation of feed (see McCarthy *et al.*, 1993). X-rays were taken using a Philips Practix, type XB 1021/00 machine at a distance of 1m from the fish. The number of Ballotini beads in the stomach of each fish was recorded from the developed X-rays. 417 pellets were used to provide a calibration curve of the number of Ballotini beads per given weight of feed ($R^2 = 0.92$, $F_{1,89} = 1012.72$, $P < 0.001$). This equation allowed calculation of amount of feed consumed. All pellets contained Ballotini, and there was an average of 10 Ballotini beads per pellet.

2. 2. 7 Injuries

When the subjects were transferred into the experimental tanks there were no visible injuries. However, the presence of minor injuries was recorded during the study as these could potentially influence performance. The injuries observed and recorded during the study included skin injuries (exacerbated by abrasion against feed collectors in three tanks) and eye injuries (believed to have been caused by abrasion against small screws on the tank windows that were present in all tanks throughout the experiment). Skin injuries were observed on the sides and ventral surface of the fish; they consisted of mild scale abrasion or small sores where the scales were missing and flesh could be seen. Eye injuries consisted of a slightly opaque film covering the eye and were usually only present on the eye that was next to the tank wall according to the direction of the water flow. Seven individuals were removed during the study as they sustained severe skin injuries; they were also removed from all data analysis. Fish that sustained eye injuries were found to have a lower growth (see results), so these subjects were omitted from the analyses of weight, growth and condition.

2. 2. 8 Growth and condition

Growth was calculated for tagged individuals using Thermal Growth Coefficient (TGC) according to the following formula (Cho, 1992):

$$TGC = ((W_{t1}^{1/3} - W_{t0}^{1/3}) / T \times t)1000$$

Where W_{t0} represents weight in grams at time 0, W_{t1} weight in grams at time 1, T the mean temperature in °C, and *t* the number of growth days.

The relative condition of each fish was calculated from the regression line of ln(weight) on ln(length), where ln is the natural log. The regression line was created from all weight and length data collected from the tanks on feeding regimes of 100% (commercial ration level) during the study. Individuals in the upper and lower modes of the weight distribution (see below) were removed from the regression as they would influence the slope of the relationship. A condition score for each fish was defined as the residual weight (for a given length) from the regression line (i.e. the difference between the observed and the expected weight). Thus, fish with heavier weights than predicted for a given length had a positive condition score, while those with lower weights than expected had a negative condition score.

2. 2. 9 Measurement of Variation

The coefficient of variation (CV) was used as a measure of variation in size and condition. Due to the presence of modes in the weight distribution (see results), CV was only used for analyses of weight variation when the upper and lower modes were excluded, leaving the main mode which had a normal weight distribution. CV was calculated using the equation below:

$$CV = (100s)/X$$

Where X is the population mean of the measured parameter and *s* is the population standard deviation of the measured parameter.

Data on feed intake and the weight of whole populations were not normally distributed. Therefore, a non-parametric equivalent of CV (CV_{np}) was used to assess variation within groups. This was calculated according to the following formula:

$$CV_{np} = IQ/ M$$

Where M is the group median of the measured parameter and IQ is the group interquartile range of the measured parameter.

2. 2. 10 Tag retention and the effects of tagging

The rate of tag retention was high; 92%, 91% and 99% of tags were retained in phase I, II and III respectively. There was no significant difference in the weight of tagged and non-tagged fish at the end of phase I, II or III (Mann-Whitney U; phase I: U = 159473.0, Z = -0.626 *P* = 0.531; phase II: Mann-Whitney U test: U = 133714.0, Z = -1.167, *P* = 0.243; phase III: independent sample t-test (used as data was normally distributed): t_{1043} = 0.082, *P* = 0.934). Nor was there a difference in the condition of tagged and non-tagged fish at the end of phase I, II or III (Mann-Whitney U test: phase I: U = 135938.0, Z = -0.311, *P* = 0.756; phase II: U = 128380.5, Z = -1.093, *P* = 0.275; phase III: U = 114411.5, Z = -1.037, *P* = 0.300).

2. 2. 11 Statistical analysis

2. 2. 11. 1 Reference table

Due to the extensive nature of the current study a reference table (Table 2.1) is provided below to facilitate understanding of questions and statistical tests used. An additional detachable reference copy is also provided.

Section	Question	Statistical test used	Page no.
2.2 METHODS			15
2.2.7 HOUSING	Did populations that were grown in tanks with feed collectors have a higher proportion of skin injuries?	Chi-square test of association on the frequency of fish with and without skin injuries in tanks with feed collectors and tanks without feed collectors	15
2.2.10 TAG RETENTION	Did VI tagging affect growth?	Weights of tagged and un-tagged fish were compared using Mann-Whitney U tests at the end of phases I and II as the data had a non-normal distribution, a t-test was used at the end pf phase III as the data was normally distributed	21
	Did VI tagging affect condition?	Mann Whitney U test comparing condition of tagged and un-tagged fish at the end of phase I, II and III	21
2.3 RESULTS			
2.3.1 WEIGHT CHANGE DURING ACCLIMATION PERIOD	Was there a difference in the weight of the 10 populations when they were transferred into the experimental tanks?	One-Way ANOVA comparing the weights of each population	29
	Was there a relationship between population weight and subsequent feeding regime when the fish were transferred to the experimental tanks?	Regression of subject weight against subsequent feeding regime	30
	Did the weight of the populations change during the acclimation period?	Mann-Whitney U test comparing start weight and weight after acclimation of all populations combined	30
	Was there a difference in the weight of the 10 populations following the acclimation period?	Kruskal Wallace test comparing weights of all tanks	30
	Was there a relationship between population weight and subsequent feeding regime following the acclimation period?	Regression of subject weight against subsequent feeding regime	30
2.3.2 VARIATION WITHIN POPULATIONS	Was the weight distribution bi-modal at any point in the study?	Visual inspection of weight distribution graphs and use of cumulative percentage frequency graphs	30
	Does weight increase throughout the study?	Series of Mann-Whitney U tests comparing weight at the start and end of each phase	31
	Is there a correlation between weight after acclimation and weight at the end of phase I?	Tagged individuals used to plot a regression of weight at the end of phase I against weight at the start of phase I	32

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Is there a correlation between weight after acclimation and weight at the lend of phase III?	Tagged individuals used to plot a regression of weight at the end of phase III against weight at the start of phase I	32
	Did weight variation of the main distribution increase throughout the study?	Repeated measures ANOVA on CVnp of each tank at each sampling	33
	Did weight variation of the whole population increase throughout the study?	Repeated measures ANOVA on CV of each tank at each sampling	33
	Is there a relationship between the population weight and weight variation without the effect of time?	Series of regressions of CV against mean weight at each sampling	34
	Was there a difference in the proportion of individuals in the lower and main modes at the start and end of phase I?	Chi-square tests of association of the proportion of fish in each mode at the end of each phase	36
	Was there a difference in the proportion of individuals in the lower, upper and main modes at the start and end of phases II and III?	Chi-square tests of association of the proportion of fish in each mode at the start and end of each phase	36
	Did the cut-off weight of the lower mode change from the start to the end of phase I?	Paired samples t-test on the cut-off weight of the lower mode for each tank at the start and end of phase I	37
	Did the cut-off weight of the lower mode change from the end of phase I to the end of phase III?	Repeated measures ANOVA on the cut-off weight of the lower mode for each tank at the end of phases I, II and III	37
	Did the cut-off weight of the upper mode change from the end of phase I to the end of phase III?	Repeated measures ANOVA on the cut-off weight of the upper mode for each tank at the end of phases I, II and III	37
	Did the cut off weights of the lower and upper modes increase as the population weight increases?	Regressions of lower and upper mode cut-off weights against the mean weight of the main distribution of each population	37
	Was there a difference in the weight, length or condition of fish that moved from the lower mode to the main mode in phase I?	Series of Mann-Whitney U tests comparing the weight, length and condition of individuals that stayed in the lower mode with those that moved from the lower mode to the main mode	39
	Was there a difference in the rate of increase of the cut-off weights of the lower and upper modes?	comparison of gradients of regressions of lower and upper mode cut-off weights against the mean weight of the main distribution of each population	38

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Was there a difference in the weight of fish that moved up or down modes in phases II and III?	Subjects in each mode in each tank were ranked by weight. A series of Mann-Whitney U tests then compared the weight- rank of individuals that moved out of each mode with those that stayed	38
	Were the fish that remained in the lower mode of the weight distribution the same fish that were in the lower mode of the growth distribution?	Chi-Square test of association comparing the frequency of individuals that were in the lower mode of the weight distribution and in the lower mode of the growth distribution with the frequency of individuals that were not in the lower mode of the weight distribution or the lower mode of the growth distribution	42
	Was there a difference in the rate of growth in each phase?	Series of Mann-Whitney U tests comparing growth rates in each phase	42
	Was there a difference in growth variation in each phase?	One-way ANOVA with *post hoc* LSD test comparing the growth variation during each phase	43
	Does initial weight, length or condition, or a combination of these predict growth? If so, which factor is the most important?	Stepwise multiple regression with growth as the dependent variable and weight, condition and length as the independent variables	44
	Did the condition of the subjects change during the study?	Repeated measures ANOVA on condition at each sampling	45
	Was the change in condition influenced by length or weight to the greatest extent?	visual inspection of graphs of length and weight plotted against time on different y-axes	46
	Did the variation in condition change during the study?	One-way ANOVA with post hoc LSD test comparing the variation in condition during each phase	46
	Was there a relationship between growth and the number of times individuals were recorded feeding?	Mann-Whitney U test comparing the growth of individuals that fed at 1, 2 or 3 samplings	47
	Was there a relationship between the total amount eaten during samplings and the total growth during the study?	Regression of total amount eaten (expressed as % bodyweight) against total growth	48
	Did the incidence of skin injuries change throughout the study?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase	48

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Did populations that were grown in tanks with feed collectors have a higher proportion of skin injuries before and after feed collectors were removed?	Chi-Square tests of association on the frequency of fish with and without skin injuries in tanks with feed collectors and tanks without feed collectors. Tests were carried out at the end of phase I (when feed collectors were present) and at the end of phase II (when feed collectors had been removed)	48
	Did the incidence of skin injuries change throughout the study in tanks without feed collectors?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase in tanks without feed collectors	48
	Did the incidence of eye injuries change throughout the study?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase	48
	Were fish that had an eye injury at one sampling more likely to have an injury at the next sampling than fish that were not originally injured?	Chi-Square test of association comparing the proportion of originally injured fish that were injured at the following sampling with the proportion of originally uninjured fish that were injured at the following sampling. Carried out separately for eye and skin injuries	50
2.3.3. EFFECT OF FEEDING REGIME	Is there a greater incidence of skin and / or eye injury in populations that grow faster?	Regression of injury incidence against mean growth rate of each population for skin and for eye injuries	50
	Do skin or eye injuries affect growth?	Series of Mann-Whitney U tests comparing the growth of injured with non-injured individuals. Tests were carried out on growth during the phase that the injury was sustained and the phase after the injury was sustained. Carried out separately for skin and eye injuries. Analysis of skin injuries was carried out separately	50
	Is there a difference in the weight of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)	Kruskall-Wallace test on the weight of fish from populations grown on 90%, 100% and 120% of commercial feed levels at the end of each phase	52
	Is there a difference in the growth of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)?	One-Way ANOVA for data from phase I and phase II and Kruskal Wallace tests on data from phase III, on the growth of fish from populations grown on 90%, 100% and 120% of commercial rations	53

Section	Question	Statistical test used	Page no.
2.3.3. EFFECT OF FEEDING REGIME	Is there a difference in the condition of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)?	One-Way ANOVA, with *post hoc* LSD test on the growth of fish from populations grown on 90%, 100% and 120% of commercial feed levels from phase I, II and III	54
	Do fish exposed to higher feeding regimes have heavier weights?	Regression of fish weight against feeding regime for each sampling	55
	Is there a relationship between feeding regime and weight variation of the whole population and of the main mode of the weight distribution?	Regression of fish weight variation against feeding regime for each sampling	56
	Does feeding regime affect the proportion of fish in each mode of the weight distribution?	Series of Chi-Square tests of association at the end of each phase comparing the proportion of individuals in each mode in low and high feeding regimes	59
	Does the proportion of fish in each mode change over time for populations reared on high or low feeding regimes?	Chi-Square tests of association comparing the frequency of fish in each mode over time. Tests carried out on low and high feeding regimes separately	59
	Is the proportion of fish moving modes from one sampling to another affected by ration?	Chi-Square test of association comparing the proportion of fish that moved from one mode to another in low and high rations	59
	Is there a difference in the growth of fish fed on low feeding regimes (30-70%) throughout the study?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is there a difference in the growth of fish fed on high feeding regimes (90-120%) throughout the study?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is growth rate higher when fish are fed on higher feeding regimes?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is growth variation affected by feeding regime?	Regression of growth variation against feeding regime for each sampling	62
	Is variation in condition affected by regime?	Regression of variation in condition against feeding regime for each sampling	63
	Is growth variation the same in each phase of the study?	Wilcoxon signed ranks test on growth in each phase	62
	Is condition affected by feeding regime?	Regression of condition against feeding regime for each sampling	63

Section	Question	Statistical test used	Page no.
2.3.3. EFFECT OF FEEDING REGIME	Do individuals exposed to low feeding regimes eat less than individuals exposed to high feeding regimes?	Regression of amount eaten (expressed as % bodyweight) against feeding regime for each sampling	65
	Was there a correlation between the amount eaten at one sampling by an individual and the amount eaten at the following sampling by the same individual?	Regression of amount eaten at the end of phase I and II (expressed as % bodyweight) against the amount eaten at the end of phase II and II, respectively	66
	Did individuals that eat more have higher growth rates? Is the result the same in high and low feeding regimes?	Regression of amount eaten (expressed as % bodyweight) against growth at the end of phase I, II and III for each sampling	66
	Did fish that grew faster eat more? Was the relationship the same in high and low feeding regimes?	Regression of growth against amount eaten (expressed as % bodyweight eaten) for each sampling	66
	Did larger fish eat more ? Is the relationship the same in low and high feeding regimes?	Regression of fish size against amount eaten (expressed as % bodyweight eaten) for each sampling	69
	Is individual variation in food intake higher in high feeding regimes than low feeding regimes?	Mann-Whitney U test comparing the variation in feed intake of fish from low and high feeding regimes at each sampling	71
	Was the distribution of feed intake more polarised in lower feeding regimes?	Individuals ranked by the mean share of the meal (MSM) they had eaten. A greater disparity in the MSM between individuals in a tank indicates greater degree of polarisation	71
	Was there an effect of regime on injury incidence?	Visual inspection of graphs detailing percentage of fish with injuries in each tank over time	71
	Was there an interaction between feeding regime and eye injury on growth?	ANOVA with feeding regime and presence or absence of eye injury	71

Section	Question	Statistical test used	Page no.
2.2 METHODS			15
2.2.7 HOUSING	Did populations that were grown in tanks with feed collectors have a higher proportion of skin injuries?	Chi-square test of association on the frequency of fish with and without skin injuries in tanks with feed collectors and tanks without feed collectors	15
2.2.10 TAG RETENTION	Did VI tagging affect growth?	Weights of tagged and un-tagged fish were compared using Mann-Whitney U tests at the end of phases I and II as the data had a non-normal distribution, a t-test was used at the end pf phase III as the data was normally distributed	21
	Did VI tagging affect condition?	Mann Whitney U test comparing condition of tagged and un-tagged fish at the end of phase I, II and III	21
2.3 RESULTS			
2.3.1 WEIGHT CHANGE DURING ACCLIMATION PERIOD	Was there a difference in the weight of the 10 populations when they were transferred into the experimental tanks?	One-Way ANOVA comparing the weights of each population	29
	Was there a relationship between population weight and subsequent feeding regime when the fish were transferred to the experimental tanks?	Regression of subject weight against subsequent feeding regime	30
	Did the weight of the populations change during the acclimation period?	Mann-Whitney U test comparing start weight and weight after acclimation of all populations combined	30
	Was there a difference in the weight of the 10 populations following the acclimation period?	Kruskal Wallace test comparing weights of all tanks	30
	Was there a relationship between population weight and subsequent feeding regime following the acclimation period?	Regression of subject weight against subsequent feeding regime	30
2.3.2 VARIATION WITHIN POPULATIONS	Was the weight distribution bi-modal at any point in the study?	Visual inspection of weight distribution graphs and use of cumulative percentage frequency graphs	30
	Does weight increase throughout the study?	Series of Mann-Whitney U tests comparing weight at the start and end of each phase	31
	Is there a correlation between weight after acclimation and weight at the end of phase I?	Tagged individuals used to plot a regression of weight at the end of phase I against weight at the start of phase I	32

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Is there a correlation between weight after acclimation and weight at the lend of phase III?	Tagged individuals used to plot a regression of weight at the end of phase III against weight at the start of phase I	32
	Did weight variation of the main distribution increase throughout the study?	Repeated measures ANOVA on CVnp of each tank at each sampling	33
	Did weight variation of the whole population increase throughout the study?	Repeated measures ANOVA on CV of each tank at each sampling	33
	Is there a relationship between the population weight and weight variation without the effect of time?	Series of regressions of CV against mean weight at each sampling	34
	Was there a difference in the proportion of individuals in the lower and main modes at the start and end of phase I?	Chi-square tests of association of the proportion of fish in each mode at the end of each phase	36
	Was there a difference in the proportion of individuals in the lower, upper and main modes at the start and end of phases II and III?	Chi-square tests of association of the proportion of fish in each mode at the start and end of each phase	36
	Did the cut-off weight of the lower mode change from the start to the end of phase I?	Paired samples t-test on the cut-off weight of the lower mode for each tank at the start and end of phase I	37
	Did the cut-off weight of the lower mode change from the end of phase I to the end of phase III?	Repeated measures ANOVA on the cut-off weight of the lower mode for each tank at the end of phases I, II and III	37
	Did the cut-off weight of the upper mode change from the end of phase I to the end of phase III?	Repeated measures ANOVA on the cut-off weight of the upper mode for each tank at the end of phases I, II and III	37
	Did the cut off weights of the lower and upper modes increase as the population weight increases?	Regressions of lower and upper mode cut-off weights against the mean weight of the main distribution of each population	37
	Was there a difference in the weight, length or condition of fish that moved from the lower mode to the main mode in phase I?	Series of Mann-Whitney U tests comparing the weight, length and condition of individuals that stayed in the lower mode with those that moved from the lower mode to the main mode	39
	Was there a difference in the rate of increase of the cut-off weights of the lower and upper modes?	comparison of gradients of regressions of lower and upper mode cut-off weights against the mean weight of the main distribution of each population	38

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Was there a difference in the weight of fish that moved up or down modes in phases II and III?	Subjects in each mode in each tank were ranked by weight. A series of Mann-Whitney U tests then compared the weight- rank of individuals that moved out of each mode with those that stayed	38
	Were the fish that remained in the lower mode of the weight distribution the same fish that were in the lower mode of the growth distribution?	Chi-Square test of association comparing the frequency of individuals that were in the lower mode of the weight distribution and in the lower mode of the growth distribution with the frequency of individuals that were not in the lower mode of the weight distribution or the lower mode of the growth distribution	42
	Was there a difference in the rate of growth in each phase?	Series of Mann-Whitney U tests comparing growth rates in each phase	42
	Was there a difference in growth variation in each phase?	One-way ANOVA with *post hoc* LSD test comparing the growth variation during each phase	43
	Does initial weight, length or condition, or a combination of these predict growth? If so, which factor is the most important?	Stepwise multiple regression with growth as the dependent variable and weight, condition and length as the independent variables	44
	Did the condition of the subjects change during the study?	Repeated measures ANOVA on condition at each sampling	45
	Was the change in condition influenced by length or weight to the greatest extent?	visual inspection of graphs of length and weight plotted against time on different y-axes	46
	Did the variation in condition change during the study?	One-way ANOVA with post hoc LSD test comparing the variation in condition during each phase	46
	Was there a relationship between growth and the number of times individuals were recorded feeding?	Mann-Whitney U test comparing the growth of individuals that fed at 1, 2 or 3 samplings	47
	Was there a relationship between the total amount eaten during samplings and the total growth during the study?	Regression of total amount eaten (expressed as % bodyweight) against total growth	48

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

Section	Question	Statistical test used	Page no.
2.3.2 VARIATION WITHIN POPULATIONS	Did the incidence of skin injuries change throughout the study?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase	48
	Did populations that were grown in tanks with feed collectors have a higher proportion of skin injuries before and after feed collectors were removed?	Chi-Square tests of association on the frequency of fish with and without skin injuries in tanks with feed collectors and tanks without feed collectors. Tests were carried out at the end of phase I (when feed collectors were present) and at the end of phase II (when feed collectors had been removed)	48
	Did the incidence of skin injuries change throughout the study in tanks without feed collectors?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase in tanks without feed collectors	48
	Did the incidence of eye injuries change throughout the study?	Wilcoxon signed ranks tests on the proportion of injured fish in each tank at the start and end of each phase	48
	Were fish that had an eye injury at one sampling more likely to have an injury at the next sampling than fish that were not originally injured?	Chi-Square test of association comparing the proportion of originally injured fish that were injured at the following sampling with the proportion of originally uninjured fish that were injured at the following sampling. Carried out separately for eye and skin injuries	50
2.3.3. EFFECT OF FEEDING REGIME	Is there a greater incidence of skin and / or eye injury in populations that grow faster?	Regression of injury incidence against mean growth rate of each population for skin and for eye injuries	50
	Do skin or eye injuries affect growth?	Series of Mann-Whitney U tests comparing the growth of injured with non-injured individuals. Tests were carried out on growth during the phase that the injury was sustained and the phase after the injury was sustained. Carried out separately for skin and eye injuries. Analysis of skin injuries was carried out separately	50
	Is there a difference in the weight of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)	Kruskall-Wallace test on the weight of fish from populations grown on 90%, 100% and 120% of commercial feed levels at the end of each phase	52

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

Section	Question	Statistical test used	Page no.
2.3.3. EFFECT OF FEEDING REGIME	Is there a difference in the growth of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)?	One-Way ANOVA for data from phase I and phase II and Kruskal Wallace tests on data from phase III, on the growth of fish from populations grown on 90%, 100% and 120% of commercial rations	53
	Is there a difference in the condition of fish reared in feeding regimes that slightly over or underfeed (with respect to commercial rations)?	One-Way ANOVA, with *post hoc* LSD test on the growth of fish from populations grown on 90%, 100% and 120% of commercial feed levels from phase I, II and III	54
	Do fish exposed to higher feeding regimes have heavier weights?	Regression of fish weight against feeding regime for each sampling	55
	Is there a relationship between feeding regime and weight variation of the whole population and of the main mode of the weight distribution?	Regression of fish weight variation against feeding regime for each sampling	56
	Does feeding regime affect the proportion of fish in each mode of the weight distribution?	Series of Chi-Square tests of association at the end of each phase comparing the proportion of individuals in each mode in low and high feeding regimes	59
	Does the proportion of fish in each mode change over time for populations reared on high or low feeding regimes?	Chi-Square tests of association comparing the frequency of fish in each mode over time. Tests carried out on low and high feeding regimes separately	59
	Is the proportion of fish moving modes from one sampling to another affected by ration?	Chi-Square test of association comparing the proportion of fish that moved from one mode to another in low and high rations	59
	Is there a difference in the growth of fish fed on low feeding regimes (30-70%) throughout the study?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is there a difference in the growth of fish fed on high feeding regimes (90-120%) throughout the study?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is growth rate higher when fish are fed on higher feeding regimes?	Repeated measures ANOVA with *post hoc* LSD test on growth rate at each sampling with tank as an factor	61
	Is growth variation affected by feeding regime?	Regression of growth variation against feeding regime for each sampling	62

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

Section	Question	Statistical test used	Page no.
2.3.3. EFFECT OF FEEDING REGIME	Is growth variation the same in each phase of the study?	Wilcoxon signed ranks test on growth in each phase	62
	Is condition affected by feeding regime?	Regression of condition against feeding regime for each sampling	63
	Is variation in condition affected by regime?	Regression of variation in condition against feeding regime for each sampling	63
	Do individuals exposed to low feeding regimes eat less than individuals exposed to high feeding regimes?	Regression of amount eaten (expressed as % bodyweight) against feeding regime for each sampling	65
	Was there a correlation between the amount eaten at one sampling by an individual and the amount eaten at the following sampling by the same individual?	Regression of amount eaten at the end of phase I and II (expressed as % bodyweight) against the amount eaten at the end of phase II and II, respectively	66
	Did individuals that eat more have higher growth rates? Is the result the same in high and low feeding regimes?	Regression of amount eaten (expressed as % bodyweight) against growth at the end of phase I, II and III for each sampling	66
	Did fish that grew faster eat more? Was the relationship the same in high and low feeding regimes?	Regression of growth against amount eaten (expressed as % bodyweight eaten) for each sampling	66
	Did larger fish eat more ? Is the relationship the same in low and high feeding regimes?	Regression of fish size against amount eaten (expressed as % bodyweight eaten) for each sampling	69
	Is individual variation in food intake higher in high feeding regimes than low feeding regimes?	Mann-Whitney U test comparing the variation in feed intake of fish from low and high feeding regimes at each sampling	71
	Was the distribution of feed intake more polarised in lower feeding regimes?	Individuals ranked by the mean share of the meal (MSM) they had eaten. A greater disparity in the MSM between individuals in a tank indicates greater degree of polarisation	71
	Was there an effect of regime on injury incidence?	Visual inspection of graphs detailing percentage of fish with injuries in each tank over time	71
	Was there an interaction between feeding regime and eye injury on growth?	ANOVA with feeding regime and presence or absence of eye injury	71

Table 2.1 Summary of questions posed and statistical tests used - an additional reference copy

2. 2. 11. 2 Weight distribution

Weight distributions were examined for evidence of modes by plotting cumulative percentage weight frequency graphs for each tank with 40 weight intervals. These graphs allowed the point at which the line deviated from the main slope to be identified by eye; this was then used as the cut-off point to identify the modal distribution of individual fish. The lower point at which the curve deviated from the main slope (x in Figure 2.2) was used as the cut-off value for identifying the lower mode and therefore the individuals within it.

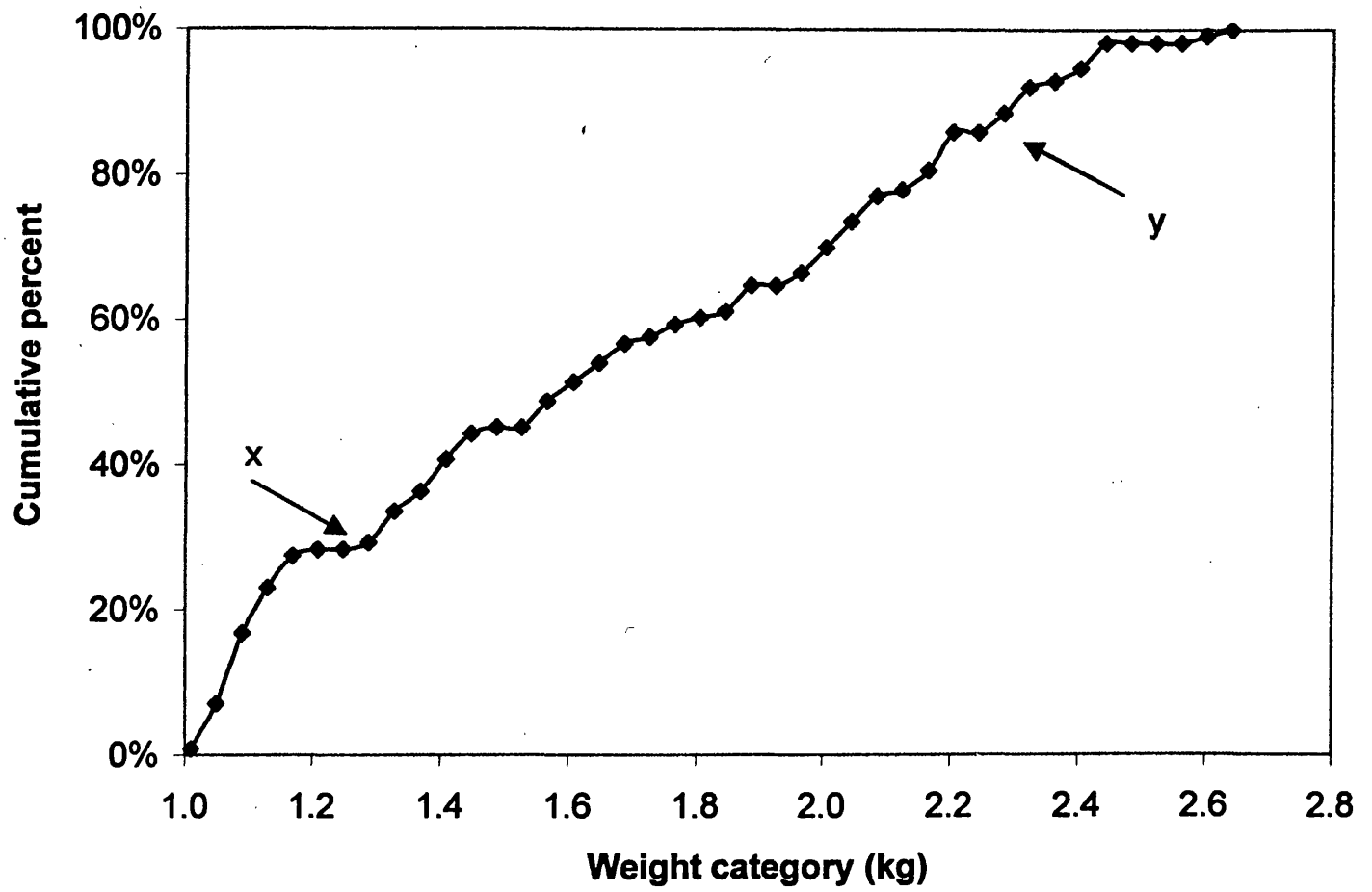


Figure 2.2 Example of cumulative percentage weight frequency graph used to identify lower mode (x) and upper mode (y).

The weight distribution of the subjects in each population was non-normal throughout the study. Removal of individuals in the lower and upper modes normalised the data, but non-parametric statistics were used in weight analysis of whole populations.

2. 2. 11. 3 Analysis of feed intake

Following conversion of feed intake data into weight of feed eaten (using the calibration curve; see above), the amount eaten was expressed as a percentage of the subjects' body weight. The amount eaten by the individuals could then be compared without the influence of size. Comparisons in the amount eaten were then made between individuals. Coefficient of variation (CV) has been used in many studies to quantify the variation in feed intake within a group (see McCarthy *et al.*, 1993; Jobling *et al.*,1995) but as the feed intake data from this study were not normally distributed a non-parametric equivalent of CV (CV_{np}) was used (see above).

The individual share of a meal (McCarthy, 1992a) was calculated for each individual at each sampling. This was calculated according to the following formula:

$$\text{share of meal} = C_{individual} / C_{population} \times 100$$

Where $C_{individual}$ is the amount eaten (in grams) by each fish and $C_{population}$ is the total amount eaten by the whole population from the sampling in question.

2. 3 RESULTS

2. 3. 1 Weight change during acclimation period

Tight size-grading ensured a low variation in weight when the fish were transferred into the tanks (CV_{np}: median and interquartile range: 4.13%, 2.1%). Weight variation increased sharply during the acclimation period (CV_{np}: median and interquartile range: 26.11, 14.14). When the fish were transferred from the sea cage to the tanks there were no differences in the weight of the 10 populations (one-way ANOVA: $F_{9,1189}$ = 1.359, P = 0.202) nor was there a relationship between subsequent feeding regime and variation in weight (CV_{np}; $F_{1,8}$ = 0.000, R^2 = 0.000, P = 0.993). However, the subjects

increased in weight during the 4 week acclimation period (Medians: initial weight = 1.31, weight after acclimation = 1.45; Mann-Whitney U test: all populations combined, U = 259841, Z = -9.907, $P < 0.001$), resulting in a marginally significant difference between the populations at the end of the acclimation period (Figure 2.3; from data collected on the subjects removed for tagging; Kruskal-Wallis test: $\chi^2 = 17.025$, d.f. = 9, $P = 0.048$). Following acclimation, fish that were to be fed on lower regimes tended to be heavier, but there was no relationship between initial weight and subsequent experimental feeding regime ($F_{1,604} = 1.804$, R-Sq = 0.03, $P = 0.180$; Figure 2.3).

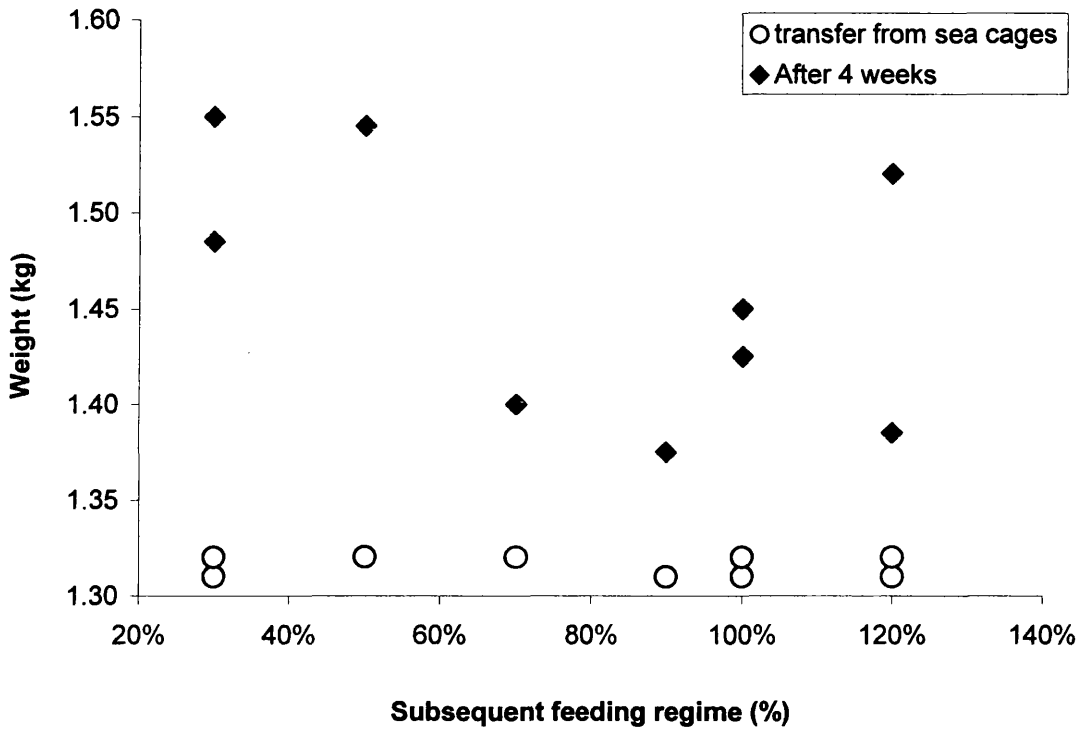


Figure 2.3 Median weights of all populations at the start (o) and end (◆) of the acclimation period, in relation to subsequent experimental feeding regimes.
N.B. Two of the 100% tanks had the same median value at transfer and after 4 weeks so there appears to be only 9 points on the figure.

The cumulative percentage weight frequency graphs indicated that there were no detectable modes when the subjects were initially transferred from the sea cage into

the experimental tanks (Figure 2.4). However, at the end of the acclimation period (during which time feeding regimes were *ad lib.*), a conspicuous lower mode of fish could be identified in the size distribution in all populations. This lower mode was evident in all populations for the remainder of the experiment. At the end of phase I (after 13 weeks), and in all remaining samplings, an upper mode, distinct from the lower mode and main distribution, was also apparent.

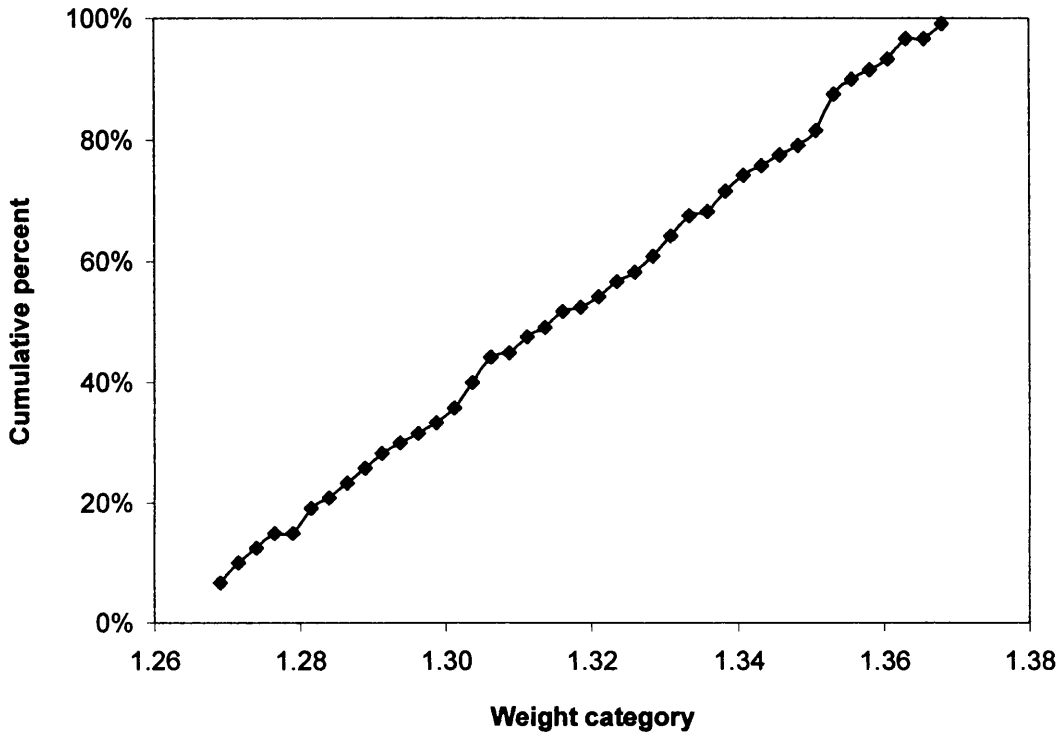


Figure 2.4 Cumulative weight frequency distribution at start of experiment, example of a size distribution with no modes.

2. 3. 2 Variation within populations

2. 3. 2. 1 Development of weight distributions

Weight trends

Weight data from all populations were combined to investigate general trends in weight increase throughout the study. This revealed a significant difference between weights

at each sampling, confirming that weight increased throughout the study (Figure 2.5; Table 2.2). The relationship between individual starting weight and weight at the end of phase I, for all subjects irrespective of feeding regime, was investigated and revealed a significant positive correlation (Figure 2.6; $F_{1,485} = 409.80$, $R^2 = 0.458$, $P < 0.001$). In addition, there was a significant positive correlation between individual starting weight and weight at the end of phase III (Figure 2.6; $F_{1,394} = 127.41$, $R^2 = 0.244$, $P < 0.001$). This indicated that in general, the largest fish after the acclimation period remained the largest fish throughout the study, irrespective of feeding regime.

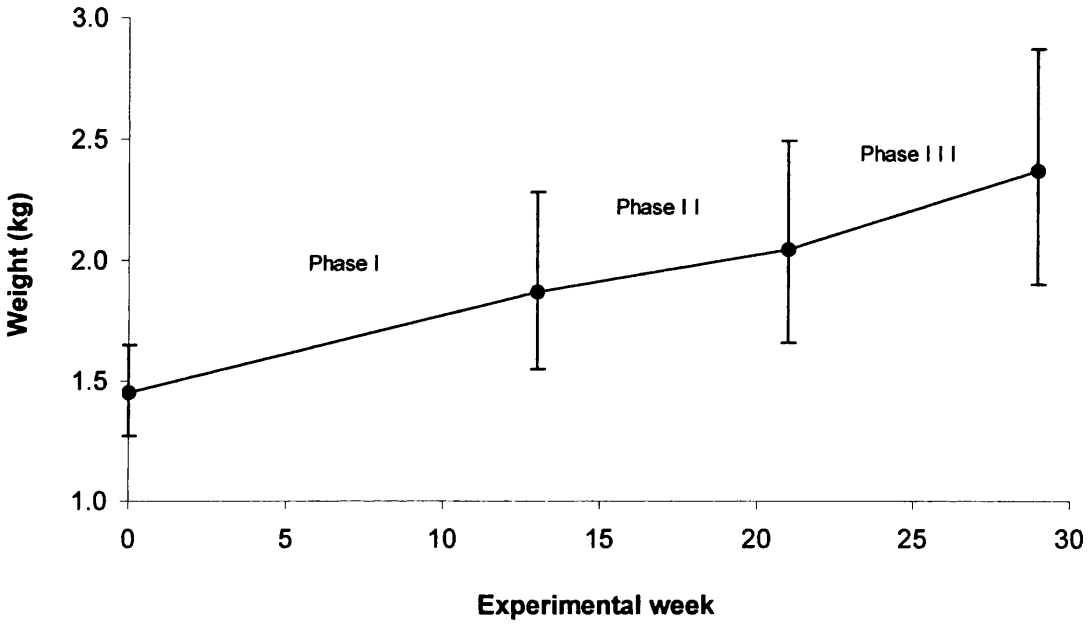


Figure 2.5 Median weight (and interquartile range) initially, and at the end of phases I, II and III.

Table 2.2 Summary of results from Mann-Whitney U tests investigating differences in weight (including all subjects) between the start and end of each phase.

	Mann-Whitney U test statistic		
Phase	U	Z	*P*
I	188320.5	-15.471	*<0.001*
II	498183.5	-6.570	*<0.001*
III	337279.0	-9.941	*<0.001*

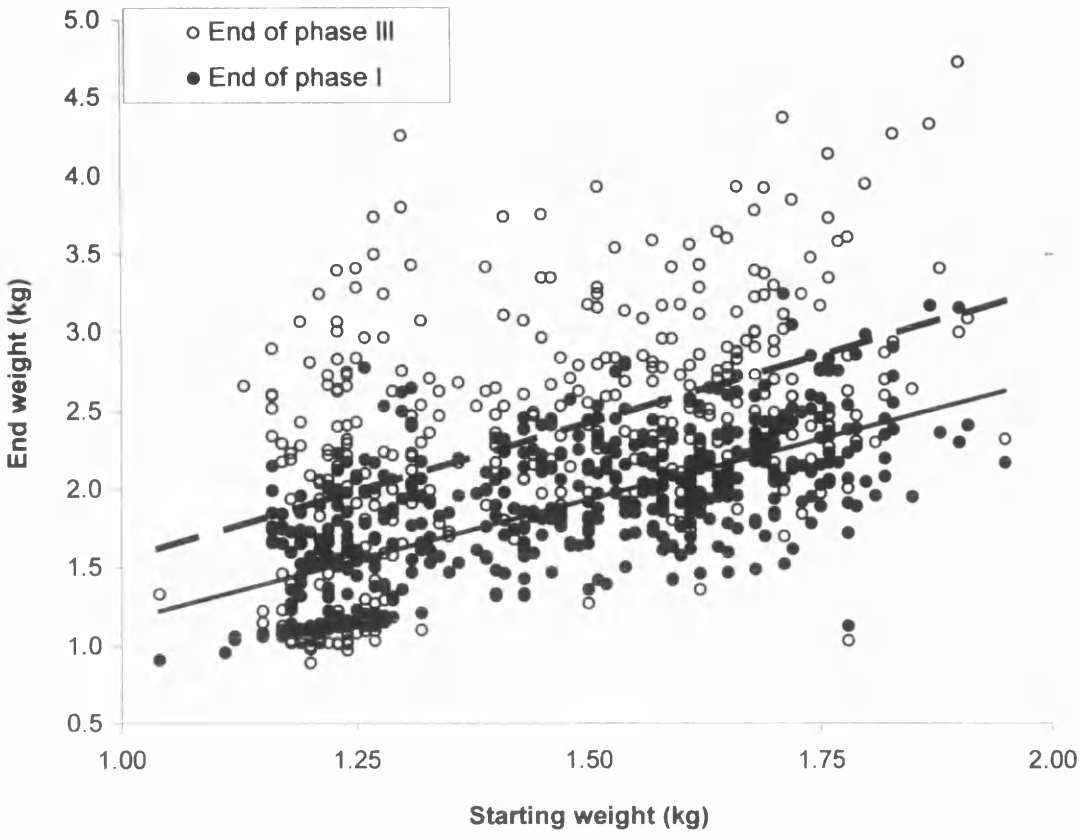


Figure 2.6 Relationship between start weight (after 4 weeks of acclimation) and weight at the end of phase I and the end of phase III.

Weight variation

Due to the presence of the upper and lower modes in the weight distribution, weight variation was initially analysed using a non-parametric equivalent for CV (CV_{np}; see methods section). A repeated measures analysis of variance (ANOVA), on the CV_{np} of each tank, revealed that weight variation changed over time (Figure 2.7; within subjects tests used as Mauchly's test of sphericity was non-significant: $F_{4,32} = 121.500$, $P < 0.001$). There was an initial rapid increase in weight variation, followed by a much slower decrease (quadratic term of polynomial contrasts: $F_{1,8} = 145.537$, $P < 0.001$). Figure 2.7 illustrates that there was an increase of 12% during the initial during phase I and a decrease of 1.1% in the remaining two phases.

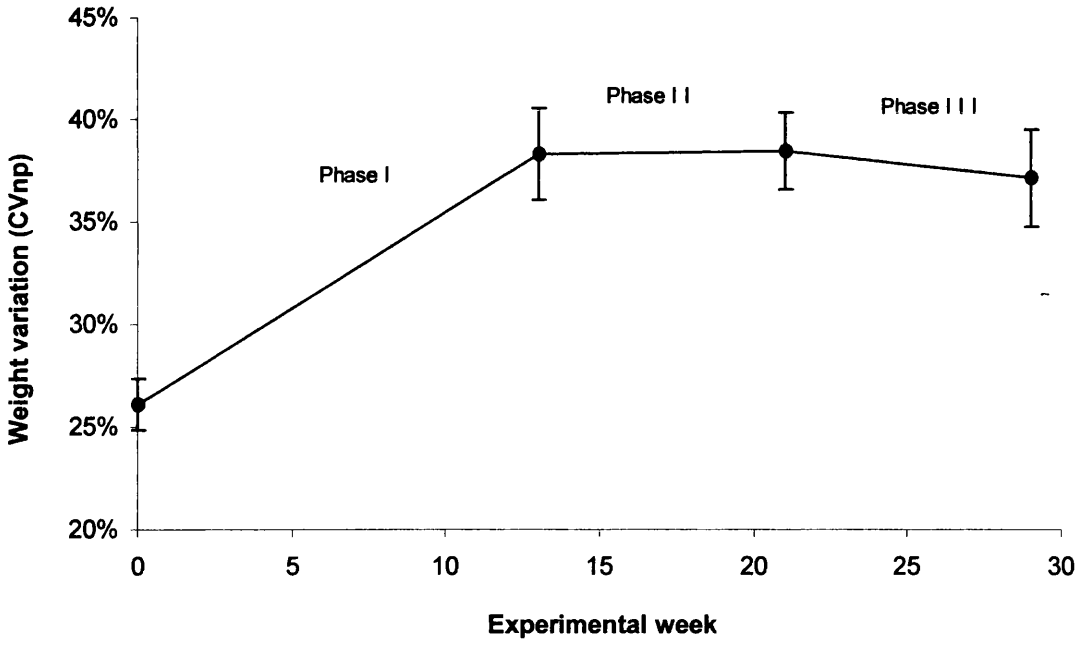


Figure 2.7 Weight variation (Mean and SE of CV_{np}) for all tanks initially, and at the end of phases I, II and III.

The main distribution (without the upper and lower modes) contained the majority of fish (74% on average). Therefore, the weight variation (CV) of the main distribution was analysed to provide information on weight variation in the main population, without the influence of the upper and lower modes. A repeated measures ANOVA revealed that there was a significant difference in the weight variation over the duration of the experiment (Figure 2.8; within subjects tests used as Mauchly's test of sphericity was non-significant, $F_{4,32} = 109.000$, $P < 0.001$). This difference represented a significant increase in weight variation throughout the study (linear term of polynomial contrasts: $F_{1,8} = 244.244$, $P < 0.001$). Figure 2.8 illustrates that there was a rapid increase during phase I (8.3%), but thereafter the increase was much slower (an increase of 1.47% over the remaining two phases). To determine if population weight affected weight variation, the mean weight was plotted against weight variation (CV) for that population. The relationship between mean weight and weight variation was investigated for each sampling separately, to remove the effect of time. This revealed that there was no

relationship between mean weight and weight variation initially, or at the end of phase I, II or III (Table 2.3).

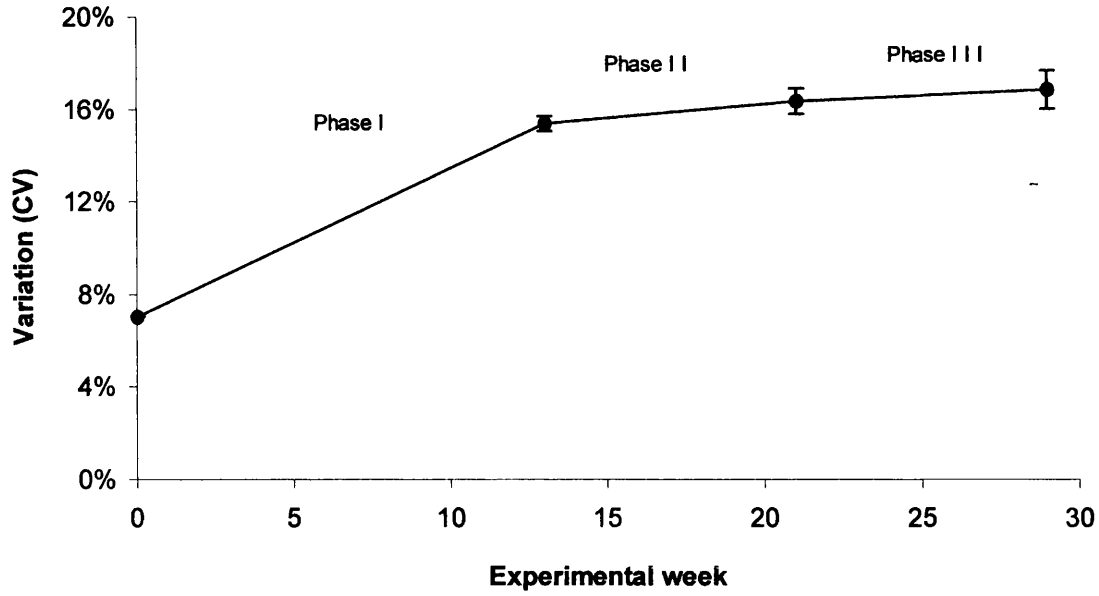


Figure 2.8 Weight variation (Mean and SE of CV) of the main mode of the size distribution initially, and at the end of phases I, II and III.

Table 2.3 Summary of results from regressions of CV on mean weight initially, and at the end of phases I, II and III.

Phase of experiment	F	R^2	*P*
Start	1.366	0.146	*0.276*
I	0.655	0.076	*0.442*
II	3.278	0.291	*0.108*
III	0.090	0.013	*0.773*

Note, d.f. = 1,8 for all regressions

Development of modes in the weight distribution

At the start of phase I (i.e. after 4 weeks of acclimation), the distribution of all populations was bi-modal, with a mean of 44% of fish found in the lower mode (Figure 2.9). By the end of phase I the distribution was tri-modal, with upper and lower modes. To compare the proportion of individuals in the lower mode initially, with the proportion at the end of each phase, the upper and main modes were combined for all populations, at the end of each phase. A chi-square test revealed that there was a significant difference between the proportion of fish in the lower and main modes initially, and the proportion of fish in the lower and main modes at the end of each phase (Figure 2.9; χ^2 = 3.012, d.f. = 3, P = 0.002). There was no difference in the proportion of fish found in the upper, lower and main modes at the end of phase I, II or III (Figure 2.9; χ^2 = 5.826, d.f. = 2, P = 0.213).

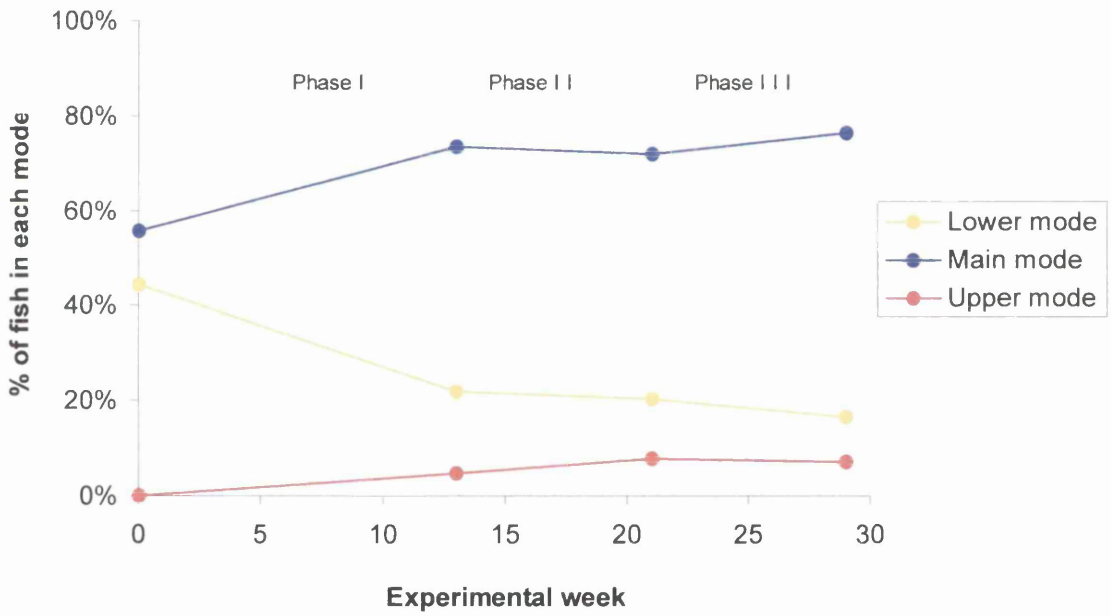


Figure 2.9 Proportion of fish in each mode in phases I, II and III (all feeding regimes combined).

A paired-samples t-test was carried out, using tanks as replicates, to test for a difference in the cut-off weight of the lower mode initially, and at the end of phase I. This revealed that cut-off weight did not change from the start to the end of phase I

(mean ± SE: start of phase I = 1.43 ± 0.014; end of phase I = 1.37 ± 0.032; $t_9 = 1.651$, $P = 0.133$).

Repeated measures ANOVA tests were then used to investigate the cut-off weights for the lower and upper modes from the end of phase I until the end of phase III, using tanks as replicates; similar results were obtained for lower and upper modes. The tests revealed that the cut-off weights for both the lower and upper modes changed over time (Figure 2.10; within subjects effects: lower mode, $F_{2,16} = 7.035$, $P = 0.006$; upper mode, $F_{2,16} = 18.627$, $P < 0.001$). The cut-off value for the both modes increased throughout the study (linear term of polynomial contrasts, lower mode: $F_{1,8} = 10.577$, $P = 0.012$; upper mode: $F_{1,8} = 23.562$, $P = 0.001$). Thus, the cut-off weights seemed to increase as the population weight increased. Indeed, when the cut-off values were plotted against the mean weights for all populations at each sampling, there was a significant positive relationship between population mean weight (of the main distribution) and the cut off value for both lower and upper modes (Figure 2.11; lower mode: $F_{1,37} = 30.148$, $R^2 = 0.449$, $P < 0.001$; upper mode: $F_{1,27} = 539.124$, $R^2 = 0.952$, $P < 0.001$).

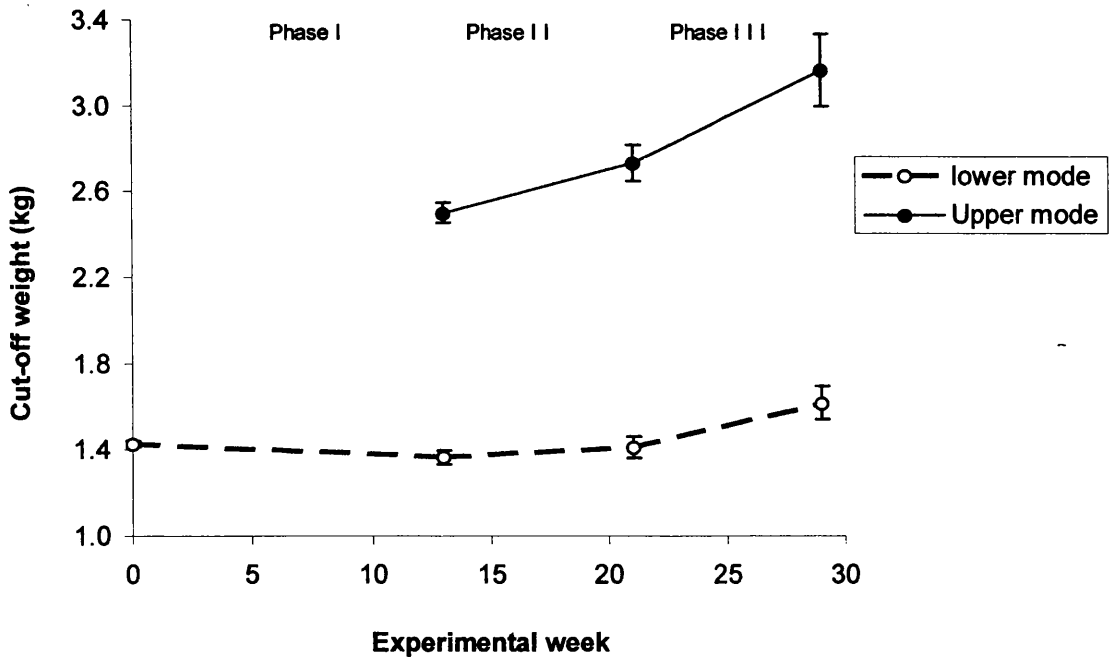


Figure 2.10 Cut-off weights of upper and lower modes, initially and at the end of phases I, II and III.

The slope of the relationship between mean weight and cut-off weight was steeper for the upper mode than for the lower mode (gradient: lower mode = 0.333; upper mode = 1.42). This indicated that the weight range between the upper and lower modes increased as the subjects increased in size. Interestingly, when the relationship between mean weight and upper mode cut-off value was extended to predict the cut-off weight for the upper mode at the start of the experiment (Figure 2.11), the lowest predicted value was higher than the heaviest observed weight at the start of the experiment. This suggested that there were no upper mode fish at the start of the experiment; actual observations were in accordance with this.

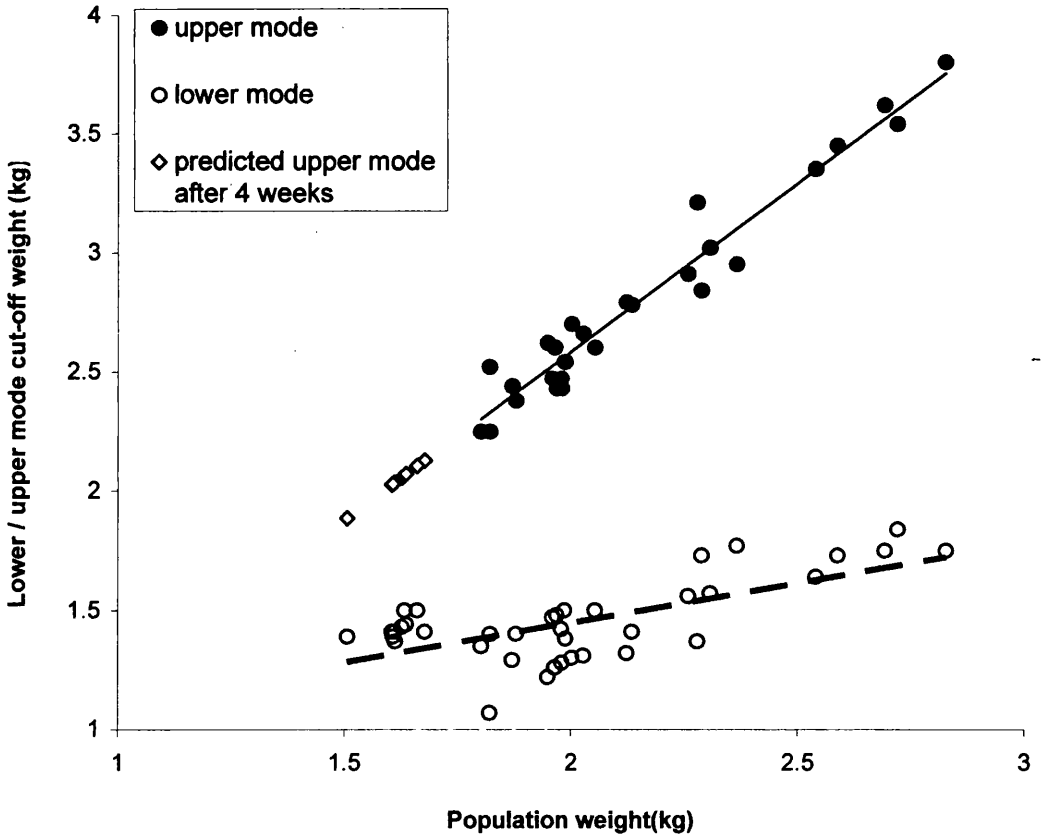


Figure 2.11 Relationship between population mean weight and the cut-off for upper and lower modes for all tanks at all sampling points.

Movement between modes in the weight distribution.

Although the majority of fish were found in the same mode of the size distribution from one sampling to the next, a small proportion of fish were found in a different mode. There were no records of individuals moving from the lower to the upper mode or from the upper to the lower mode during the study, but individuals were found to have moved one mode up or down. Combining all populations, 11% of fish moved from one mode to another in phase II and 5% moved from one mode to another in phase III.

As the weight distribution of the populations was initially bi-modal and then tri-modal for the remainder of the study, movement between modes in phase I was analysed separately from phase II and phase III. At the start of phase I, 44% of fish were found

in the lower mode, but by the end of phase I, 22% of fish were found in the lower mode. To test for differences between the subjects that moved into the main mode and those that stayed in the lower mode, a series of Mann Whitney U Tests were used to compare weight, length and condition of subjects that moved with those that stayed. These tests indicated that fish that moved into the main mode were initially heavier and longer than those that remained in the lower mode in phase I (Table 2.4). In contrast, there was no difference in the condition of fish that moved into the main mode or those that remained in the lower mode (Table 2.4).

Table 2.4 Summary of results from a series of Mann-Whitney U tests to compare the weight, length and condition of fish that stayed in the lower mode with those that moved from the lower mode to the main mode in phase I.

	Median and interquartile range		Mann-Whitney U test results		
Variable	lower mode	Main mode	U	Z	*P*
Weight	1.21kg, 0.06	1.26kg, 0.100	3549.00	-5.547	*< 0.001*
Length	46.5cm, 1.38	47.0cm,1.25	4105.00	-4.375	*< 0.001*
Condition	0.155, 0.1075	0.150, 0.1200	596.00	-1.028	*0.304*

The sample size of subjects moving from one mode to another within each tank was small in phases II and III. Therefore, the effect of initial weight on movement was analysed by ranking the fish in each mode in each population by weight. A series of Mann-Whitney U tests were then used to determine if fish that moved (up or down) from one mode to another had a different weight-rank from those that remained in the same mode. Tests were carried out separately to compare the weight-rank of fish that moved or stayed in each mode. The tests revealed that subjects that moved from the lower mode to the main mode were larger than those that remained in the lower mode. This result was found in phase I and phase II (Phase I: mean ranks: stay = 39.13, move = 60.82; U = 189.00, Z = -2.787, P = 0.005; phase II: mean ranks: stay = 29.06, move = 43.31; U = 178.00, Z = -2.504, P = 0.012).

No fish moved from the main mode into the upper mode in phase II. However, comparison of the weight-ranks of fish that moved from the main mode into the upper mode in phase III revealed that fish that moved into the upper mode had higher weight-ranks than those that remained in the main mode (mean ranks: stay = 136.40, move = 251.15; U = 243.50, Z = -4.401, $P < 0.001$). In contrast, subjects that moved from the main mode to the lower mode had significantly lower weight-ranks than those that remained in the main mode in phase II (mean ranks: stay = 178.00, move = 23.61; U = 167.50, Z = -4.558, $P < 0.001$). The sample size in phase III was too small to allow a comparison.

Finally, there was no difference in the weight-rank of subjects that moved from the upper mode to the main mode in phase II (mean ranks: stay = 13.89, move = 14.25; U = 74.00, Z = -0.108, $P = 0.938$). However in phase III, fish that moved from the upper mode to the main mode did have significantly smaller weight-ranks than those that remained in the upper mode (mean ranks: stay = 20.85, move = 12.54; U = 72.50, Z = -2.293, $P = 0.021$. In summary, subjects that moved up from one mode to another tended to be larger than subjects that remained in the same mode. Furthermore, fish that moved down from one mode to another tended to be smaller than fish that remained in the same mode.

Growth trends

At the end of phase I, the distribution of growth rates was similar to the distribution of weight, as a lower mode of fish was apparent in the growth rate distribution. However, there was no clear indication of growth modality for the remainder of the experiment. Combining all tanks, a mean of 19% of fish were found in the lower mode of the growth rate distribution during phase I. The cut-off value for the lower mode was a growth rate of zero. Therefore, there was a distinct group of fish that did not grow during phase I. A chi-square test of association indicated that these fish were the same individuals that

were found in the lower mode of the weight distribution (χ^2 = 33.471, d.f. = 1, P < 0.001).

A series of Mann-Whitney U tests were used to compare the overall growth rates in each phase of the study. The tests revealed that there was a difference in growth between phase I, II and III (Table 2.5; Figure 2.12); the lowest growth rates were found in phase II and the highest growth rates in phase III. Incidentally, there was no obvious relationship between temperature or daylength and mean growth rate, as temperature decreased throughout the study and daylength increased throughout the study (Figure 2.1).

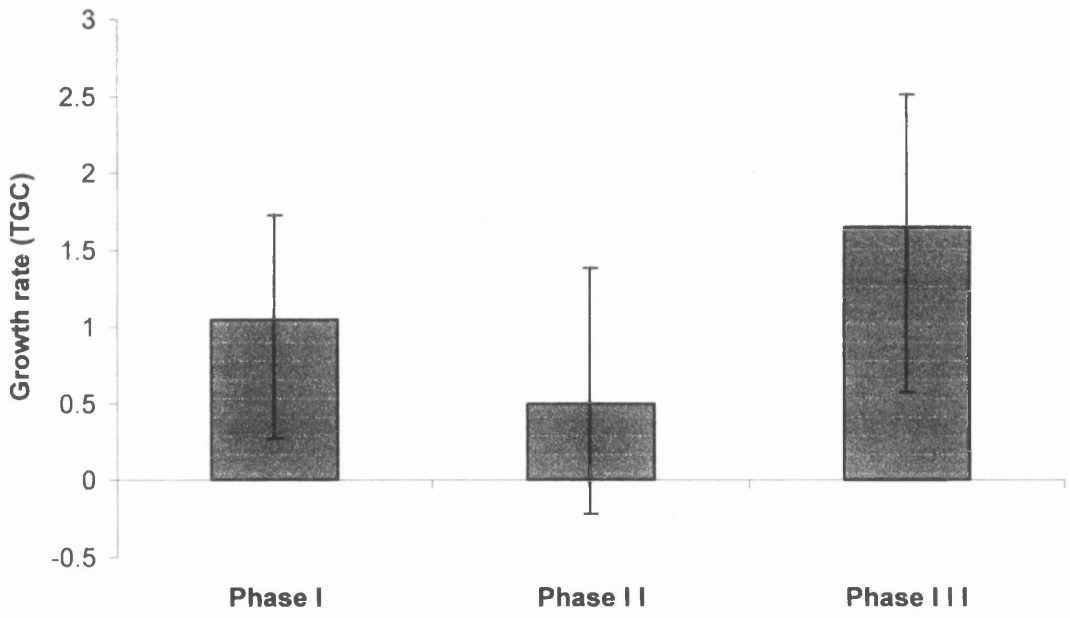


Figure 2.12 Overall growth rate during each phase.

Table 2.5 Summary of results from Mann-Whitney U tests investigating differences in growth in phases I, II and III.

Phases between which growth was compared	Mann-Whitney U test statistic		
	U	Z	*P*
I and II	91131.0	-4.578	*<0.001*
II and III	54549.0	-9.451	*<0.001*
I and III	66922.0	-6.912	*<0.001*

Variation in growth

To test for variation in growth rate over time, a one-way ANOVA with LSD *post-hoc* test was carried out on CV of growth rate (ln transformed). The lower mode was not included in the data from phase I. There was a difference in growth variation during the study (Figure 2.13; $F_{2,28} = 6.841$, $P = 0.004$), with variation in growth rate increasing as growth rate decreased. In addition, the LSD *post-hoc* test revealed that there was a significant difference between phase I and II and phase II and III, but no difference between phase I and III.

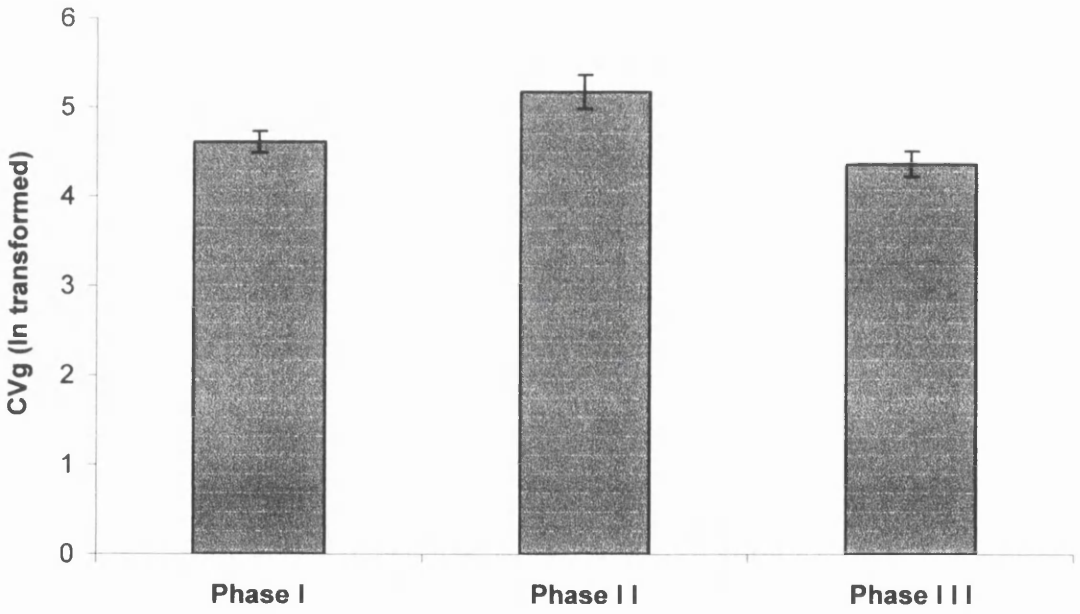


Figure 2.13 Growth variation (CV) for each phase.

Predictors of growth

To test for the relative importance of initial condition, initial weight (ln transformed) and initial length on growth in phase I, a stepwise multiple regression was performed (Table 2.6). This revealed that initial weight was the most important single explanatory variable, explaining 71% of the variation in growth. The effect of initial weight was independent of that of initial length and initial condition, but length and condition did also explain significant amounts of the remaining variation in growth. The relationship between initial weight and growth was positive, but there was a negative relationship between length and condition and the remaining variation in growth. The effects of the same variables were examined with respect to growth over the whole study using stepwise multiple regression on the overall growth rate (Table 2.6). This also revealed that initial weight was the most important single explanatory variable; explaining 50% of the variation in growth. Similarly, the effect of weight was independent of that of length and condition, but they also explained significant amounts of the remaining variation in growth with a negative relationship.

Table 2.6 Summary of results from stepwise multiple regressions for the relative influence of weight, length and condition on growth in phase I and growth over the whole experiment.

Phase	Variable	Order	T	*P*
Phase I	Weight	1	11.702	< *0.001*
	Condition	2	-9.596	< *0.001*
	Length	3	-4.800	< *0.001*
Whole experiment	Weight	1	26.613	< *0.001*
	Condition	2	-13.858	< *0.001*
	Length	3	-9.459	< *0.001*

Overall, phase I: $R^2 = 0.593$, $F_{3,411} = 201.694$; whole experiment: $R^2 = 0.915$, $F_{3,483} = 827.337$.

2. 3. 2. 2 Condition

Unlike weight, there was no evidence of lower or upper modes in the condition score at any point in the study. Initially, a repeated measures ANOVA revealed that condition changed over time (Figure 2.14; Multivariate tests used as Mauchly's test of sphericity was significant: $F_{3,402} = 836.824$, $P < 0.001$). This difference represented a marked decrease in condition over time (Linear term of polynomial contrasts: $F_{1,404} = 1816.217$, $P < 0.001$). Examination of the relative changes of length and weight during the study (Figure 2.15) revealed that the decrease in condition was due to a relative increase in length during phase I.

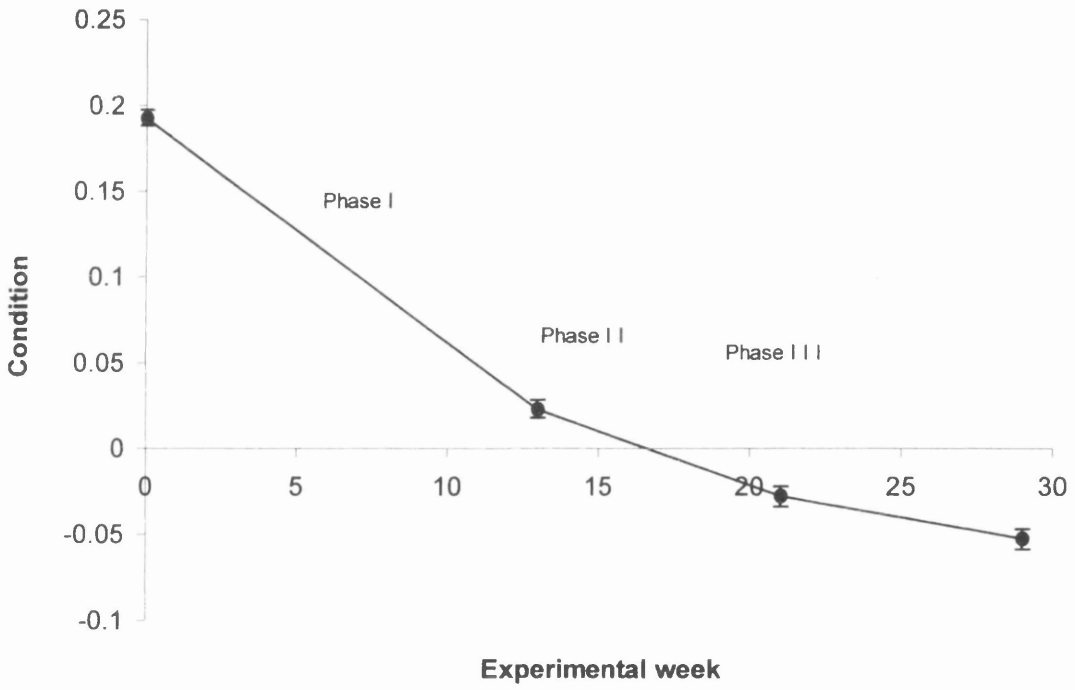


Figure 2.14 Condition (Mean and SE) initially, and at the end of phases I, II and III.

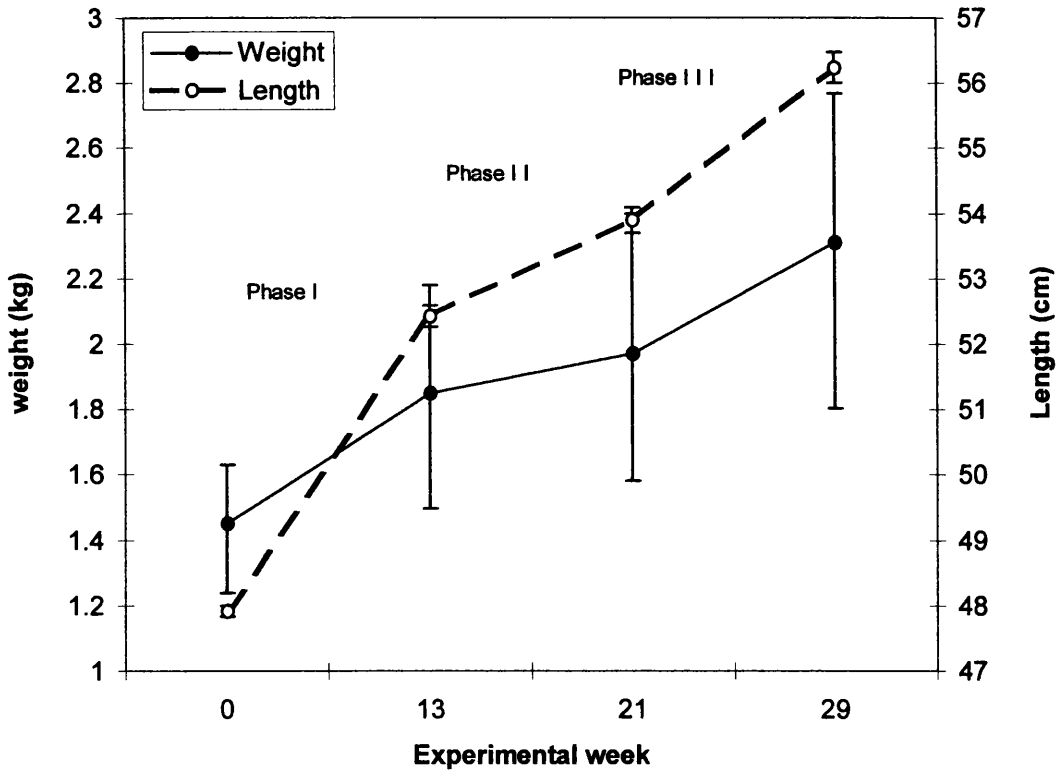


Figure 2.15 Weight and length initially, and at the end of phases I, II and III.

A one-way ANOVA with *post-hoc* lowest significant difference (LSD) test (on ln transformed data) indicated that the degree of variation in condition (CV) changed over the course of the study (Figure 2.16; $F_{3,25} = 125.905$, $P < 0.001$). The *post-hoc* LSD test indicated that there was a significant difference in variation between all samplings except the end of phase II and the end of phase III. Figure 2.16 illustrates an initial increase in variation during phase I, followed by a rapid decrease in phase II to a level lower than the original.

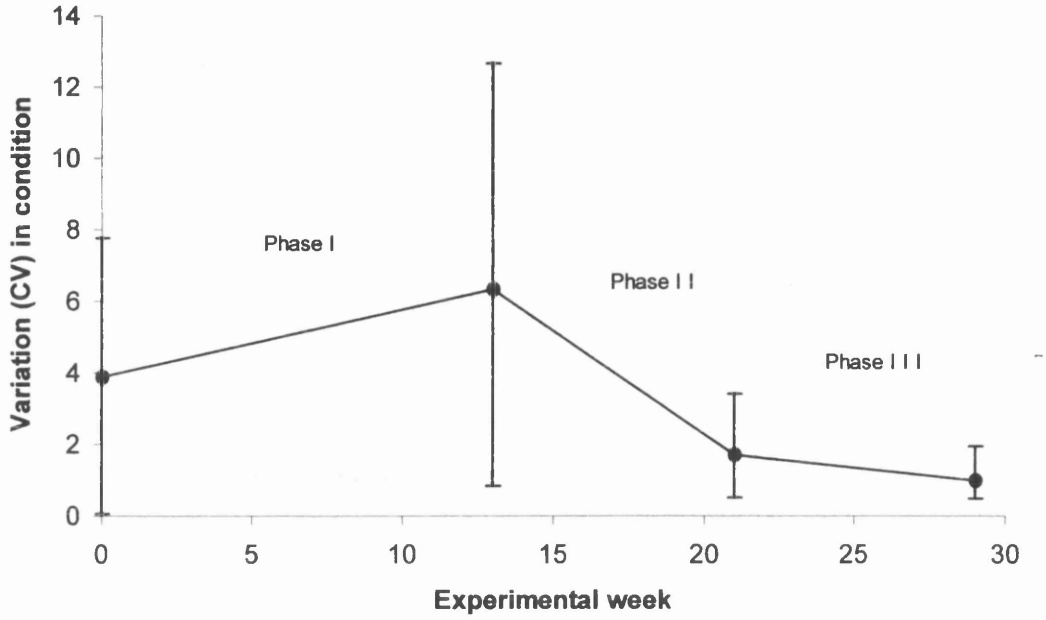


Figure 2.16 Variation in condition factor (ln transformed CV) initially, and at the end of phases I, II and III.

2. 3. 2. 3 Food Intake

The proportion of fish that had eaten was low in the first two samplings; 28% and 43% of X-rayed subjects were found to have food in their stomachs at the end of phase I and phase II respectively. The length of time that the feed was presented was increased for the third sampling and resulted in 60% of X-rayed individuals showing evidence of feeding.

Initially the data were examined for general relationships between growth rate (TGC) and feed intake. Firstly, a Mann-Whitney U test was carried out to determine if fish that had eaten at every sampling had higher growth rates than those that had not eaten at any sampling. As expected, it revealed that there was a difference in the growth rates of fish that had eaten and those that had not. Fish that had eaten had higher growth rates than those that had not (median and interquartile range: had eaten = 1.651, 0.997; had not eaten = -0.014, 0.987; U = 44.000, Z = -3.820, $P < 0.001$). Secondly,

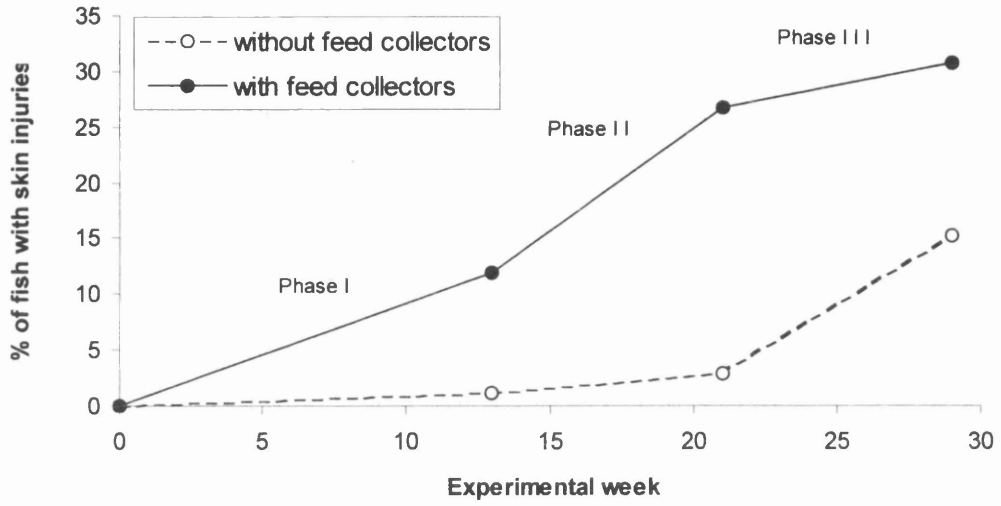
the total amount eaten over the whole study was calculated, by adding the percentage bodyweight eaten at each sampling, and plotted against total growth rate for each individual. Perhaps surprisingly, there was no significant relationship between overall amount eaten and overall growth ($F_{1,157} = 3.468$, $R^2 = 0.015$, $P = 0.064$), although there was a trend for larger fish to eat more.

2. 3. 2. 4 Injuries

Skin injuries

There was a significant increase in the incidence of skin injuries observed at each sampling (Figure 2.17). A Wilcoxon signed ranks test revealed that the incidence of skin injury differed between all samplings (phase I: Z = -2.533, $P = 0.011$; phase II: Z = -2.366, $P = 0.018$; phase III: Z = -2.395, $P = 0.017$). In addition, chi-square tests indicated significantly higher incidences of injury in tanks with feed collectors present (Figure 2.17; phase I: $\chi^2 = 65.198$, d.f. = 1, $P < 0.001$; phase II: $\chi^2 = 132.363$, d.f. = 1 $P < 0.001$); this difference persisted after the collectors were removed (phase III: $\chi^2 = 31.318$, d.f. = 1, $P < 0.001$). Therefore, individuals from tanks with feed collectors were removed from all analyses over time. Wilcoxon signed ranks tests revealed significant increases in injuries between the start and the end of phase I and between the start and end of phase III, but there were no difference between the start and end of phase II (phase I: Z = -2.060, $P = 0.039$; phase II: Z = -1.826, $P = 0.068$; phase III: Z = -2.366, $P = 0.018$).

a) Skin injuries



b)Eye injuries

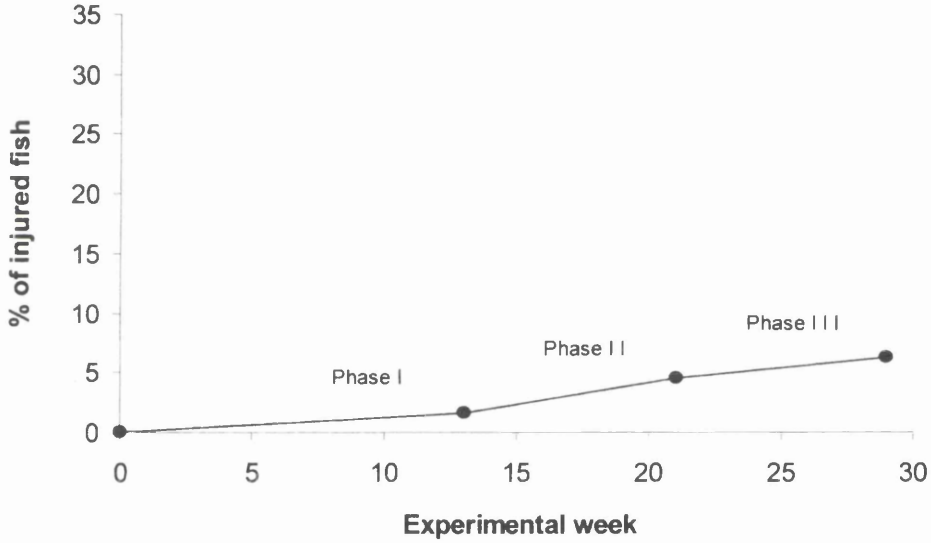


Figure 2.17 Incidence of minor injuries during the study: a) skin injuries in tanks with and without feed collectors; and b) eye injuries in all tanks.

Eye injuries

The incidence of eye injuries was much lower than the incidence of skin injuries (Figure 2.17 a & b), but the proportion of subjects with eye injuries also changed throughout the experiment. A Wilcoxon's signed ranks test revealed that the incidence increased during phase I, but there was no difference in the injury incidence in the subsequent

phases (phase I: Z = -2.371, P = 0.018; phase II: Z = -1.478, P = 0.139; phase III: Z = -0.714, P = 0.475; Figure 2.17 b).

Likelihood of continuing injury

As 50% of the fish were individually tagged, it was possible to track the occurrence of injuries on particular individuals over time. To determine the likelihood of a fish that was injured at one sampling being injured at the next sampling, a chi-square test was carried out to compare injury incidence at time one to injury incidence at time two for the same individuals. As the sample size was too small at the end of phase I, this test was carried out on injuries at the end of phase II and phase III. Individuals with skin injuries at the end of phase II were no more likely to have skin injuries at the end of phase III than individuals that did not have injuries at the end of phase II (χ^2 = 2.958, P = 0.085). In contrast, an individual with an eye injury at the end of phase II was more likely to have an eye injury at the end of phase III (χ^2 = 57.515, P < 0.001). The calculation of odds ratios (Sokal & Rohlf, 2000) from these data revealed that individuals with eye injuries at the end of phase II were 24 times more likely to have eye injuries at the end of phase III than individuals that did not have eye injuries at the end of phase II.

Injury and growth

To determine if individuals in populations that grew faster sustained more injuries, the proportion of fish injured and the mean growth rate for each tank at each sampling were plotted; this revealed that there was no relationship between growth and the incidence of eye injuries ($F_{1,27}$ = 0.642, R^2 = 0.023, P = 0.430) or skin injuries ($F_{1,27}$ = 0.984, R^2 = 0.035, P = 0.330).

In order to investigate whether the injury affected individual growth before and / or after recording of the injury. The growth of individuals that sustained injuries in the phase that it was first sustained and the subsequent phase was compared to individuals

without injuries. Analysis of the effect of skin injuries on growth was carried out separately for individuals grown in tanks with feed collectors due to the increased incidence of skin injuries in these tanks. There was no difference in the growth of fish with skin injuries (from tanks with or without feed collectors) in the phase following, or the phase preceding, the recording of the injury (Tables 2.7 a, b & c). However, there was a difference in the growth rate of fish with eye injuries and those without, although the effect was unpredictable. Fish that were injured in phase I grew slower than those that were not. However, fish that were injured in phase II showed no difference in growth in that phase, but grew slower in phase III. In conclusion, eye injuries did affect growth, but it was not clear whether they affected the growth of the individual in the phase that they were sustained, or in the following phase.

Table 2.7 Summary of results from Mann-Whitney U tests carried out to determine if there was a difference in the growth rates of fish with and without injuries in the phases preceding and following the recording of the injury a) skin injuries in fish from tanks without feed collectors and b) skin injuries in fish from tanks with feed collectors and c) eye injuries in all tanks.

a) Skin injuries in tanks with feed collectors

End of phase that	phase that injury occurred			phase following injury		
injury was recorded	U	Z	*P*	U	Z	*P*
Phase I	1061.0	-1.351	*0.177*	1185.0	-0.557	*0.577*
Phase II	1832.0	-1.517	*0.129*	1801.0	-1.320	*0.187*
Phase III	6219.0	-0.214	*0.831*			

b) Skin injuries in tanks without feed collectors

End of phase that injury was recorded	phase that injury occurred			phase following injury		
	U	Z	*P*	U	Z	*P*
Phase I	921.0	-0.781	*0.450*	798.0	-0.755	*0.450*
Phase II	1674.0	-0.377	*0.706*	418.0	-0.855	*0.393*
Phase III	241.0	-1.249	*0.212*			

c) Eye injuries in all tanks

End of phase that injury was recorded	phase that injury occurred			phase following injury		
	U	Z	*P*	U	Z	*P*
Phase I	440.0	-2.019	*0.043*	934.0	-0.143	*0.887*
Phase II	3126.0	-0.215	*0.830*	1158.0	-3.727	*0.000*
Phase III	1908.0	-1.916	*0.055*			

2. 3. 3 Effect of feeding regime

2. 3. 3. 1 Near–commercial regimes

Initially, the relationship between feeding regimes that represented under-feeding (90%), normal feeding (100%) and over-feeding (120%) of commercial feeding regimes was examined for differences in weight, condition and growth. A Kruskal-Wallis test was used to test the null hypothesis that there was no difference in the weight of fish from these tanks. The null hypothesis was not rejected; there was no difference in the weight of fish from these tanks after the end of phase I or phase II (Figure 2.18; phase I: $\chi^2 = 3.566$, d.f. = 5, $P = 0.613$; phase II: $\chi^2 = 9.147$, d.f. = 5, $P = 0.103$). There was however, a difference at the end of phase III ($\chi^2 = 10.396$, d.f. = 5, $P = 0.034$), but Figure 2.18 shows that the greatest weight difference was between the individuals grown on the 90% and 100% feeding regimes. Interestingly the weights of fish from the 120% feeding regimes lay between those fed on 90% and 100%. In conclusion, within the range of near-commercial feeding regimes there was no clear effect of over or underfeeding on weight.

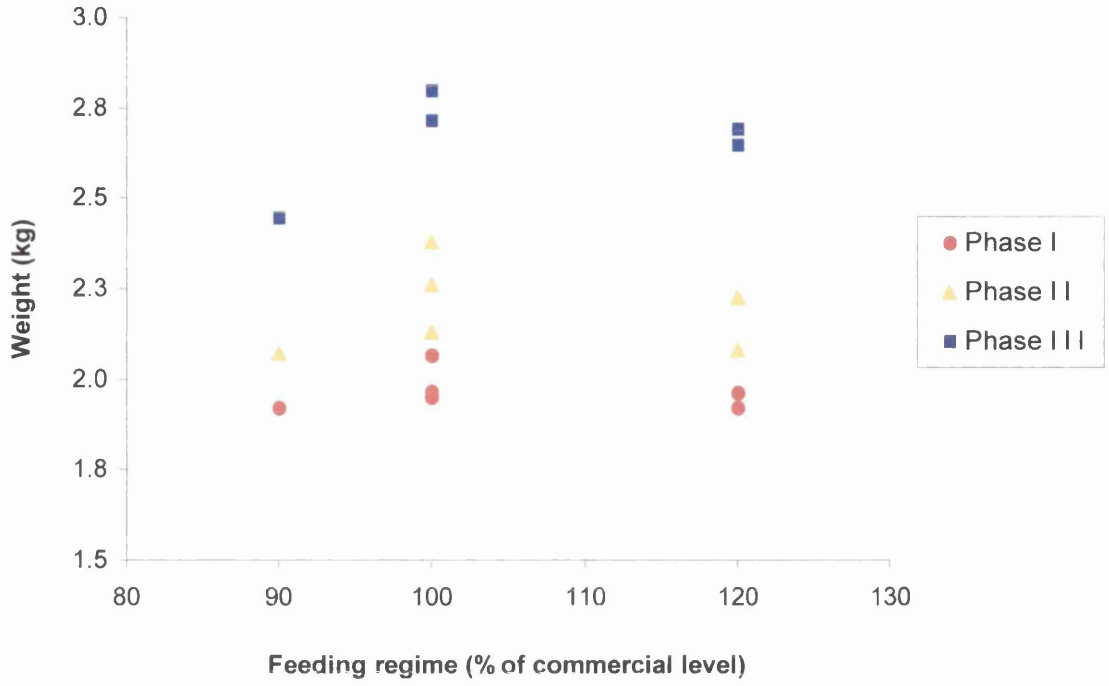


Figure 2.18 The relationship between feeding regime and median weight of populations grown on near-commercial feeding regimes, after phases I, II and III.

Analysis of growth differences in the near-commercial regimes revealed a similar trend. There was no difference in growth during phase I or phase II (One-way ANOVA: phase I, $F_{5,283} = 0.983$, $P = 0.428$; phase II, $F_{5,276} = 0.985$, $P = 0.427$), but there was a difference in the growth rates of the populations during phase III (Figure 2.19; Kruskal-Wallis test: $\chi^2 = 11.910$, d.f. = 4, $P = 0.018$). Figure 2.19 illustrates that there was a large difference between the two populations grown on 120% feeding regimes. Therefore, there was no clear difference between under-feeding, over-feeding and normal feeding levels in terms of growth.

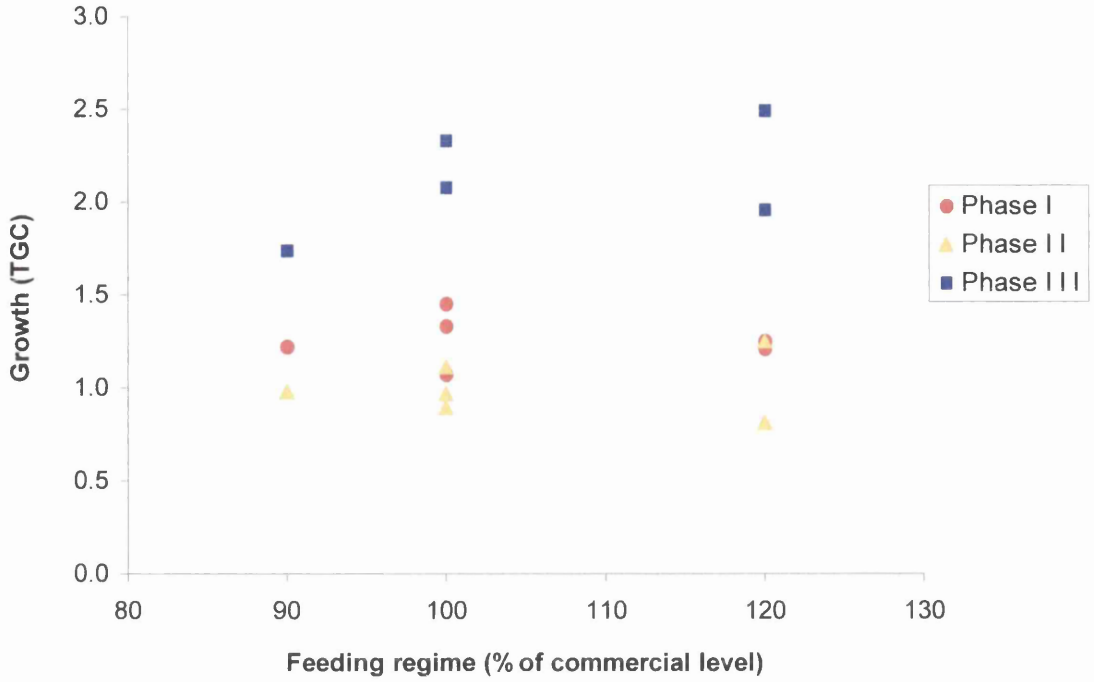


Figure 2.19 The relationship between feeding regime and growth for populations fed near-production feeding regimes over phases I, II and III.

Lastly, a one-way ANOVA was carried out to look for differences in condition amongst the populations on near-commercial feeding regimes. Although there were significant differences between the condition of the commercial comparison tanks at the end of phase I and phase III (Figure 2.20; one-way ANOVA with LSD *post-hoc* test: phase I: $F_{5,679} = 4.705$, $P < 0.001$; phase III: $F_{4,487} = 3.456$, $P = 0.008$), there were no trends in these differences as there were differences in the replicates, nor was there a difference present at the end of phase II (one-way ANOVA with LSD *post-hoc* test: $F_{5,628} = 1.558$, $P = 0.170$).

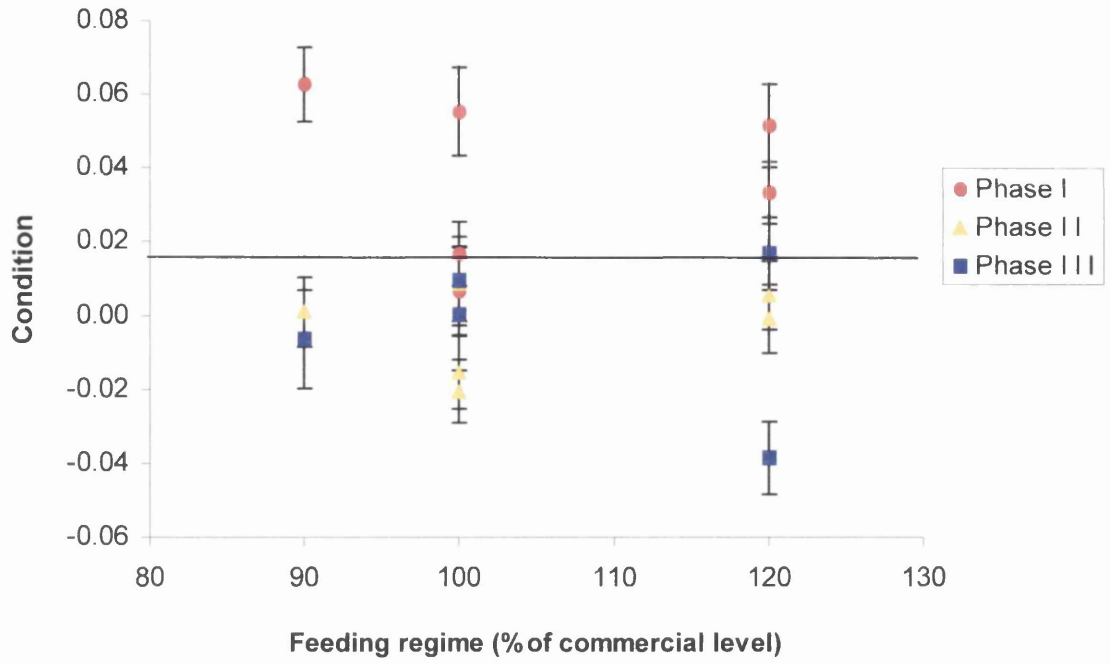


Figure 2.20 The relationship between feeding regime and condition (mean and SE) for populations grown on near commercial feeding regimes after phases I, II and III.

2. 3. 3. 2 All feeding regimes

Weight change

As expected, there was a significant positive relationship between feeding regime (expressed as a percentage of commercial feed levels) and weight at the end of each phase; individuals fed on higher feeding regimes were heavier (Figure 2.21; phase I: $F_{1,1142} = 68.264$, R-Sq = 0.056, $P < 0.001$; phase II: $F_{1,1038} = 111.062$, R-Sq = 0.096, $P < 0.001$; phase III: $F_{1,878} = 226.444$, R-Sq = 0.205, $P < 0.001$).

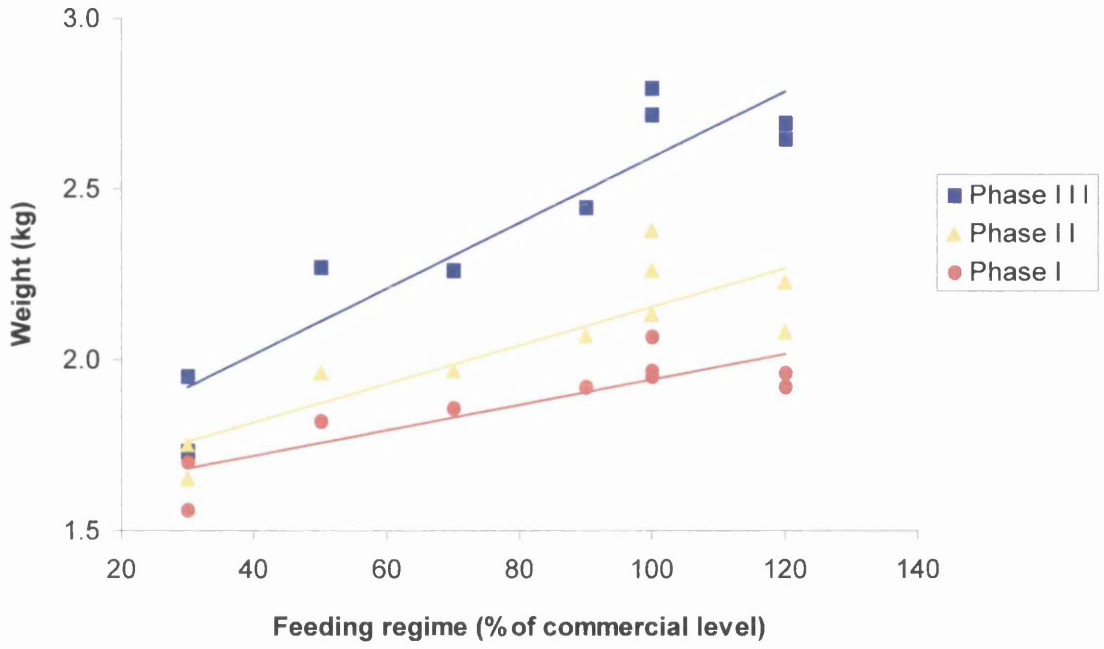


Figure 2.21 The relationship between feeding regime and median weight at the end of phases I, II and III.

Weight variation

There was a rapid increase in variability of weight upon introduction of the subjects into the tanks that persisted throughout the study (Figure 2.22). This increase was apparent in both high and low feeding regimes, but there was a difference in the weight distribution (see below). The effect of feeding regime on weight variation was investigated for the main mode of the populations (using CV) and the entire populations (using CV_{np}), by plotting a regression of variation against ration for each sampling. There was no relationship between feeding regime and weight variation in the main mode of the population at any point in the study (Table 2.8). In contrast, a negative relationship was observed between feeding regime and weight variation at the end of phases I and II in the whole population (Figure 2.23; Table 2.8). However, there was no relationship between feeding regime and weight variation in the whole population at the end of phase III.

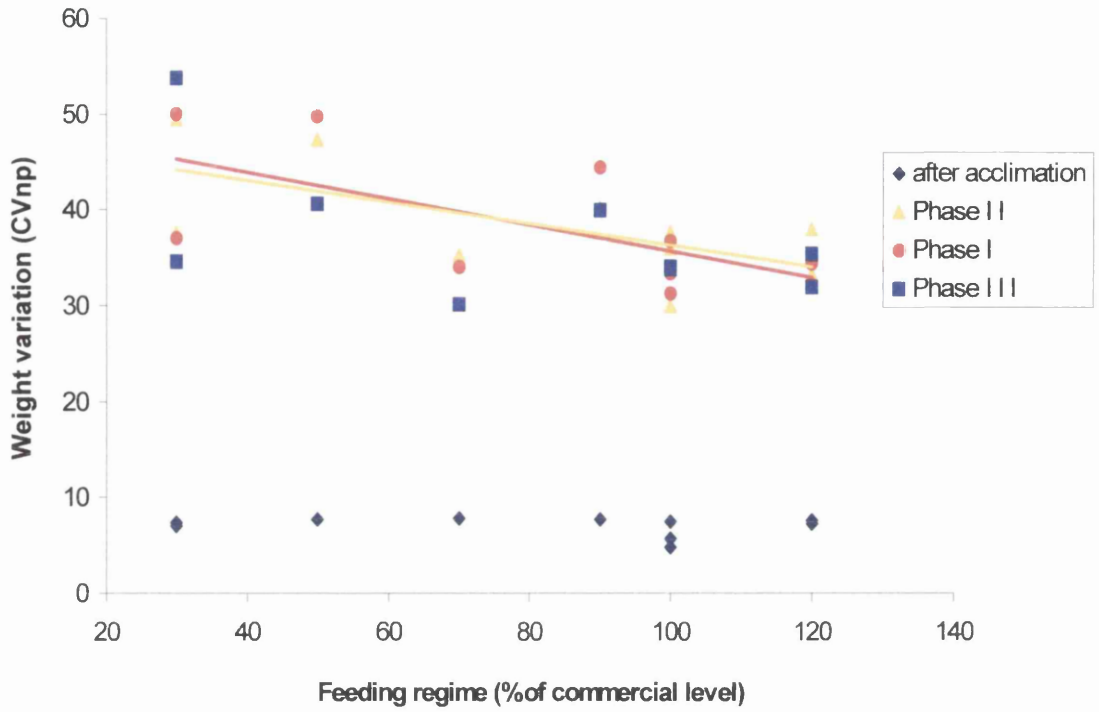
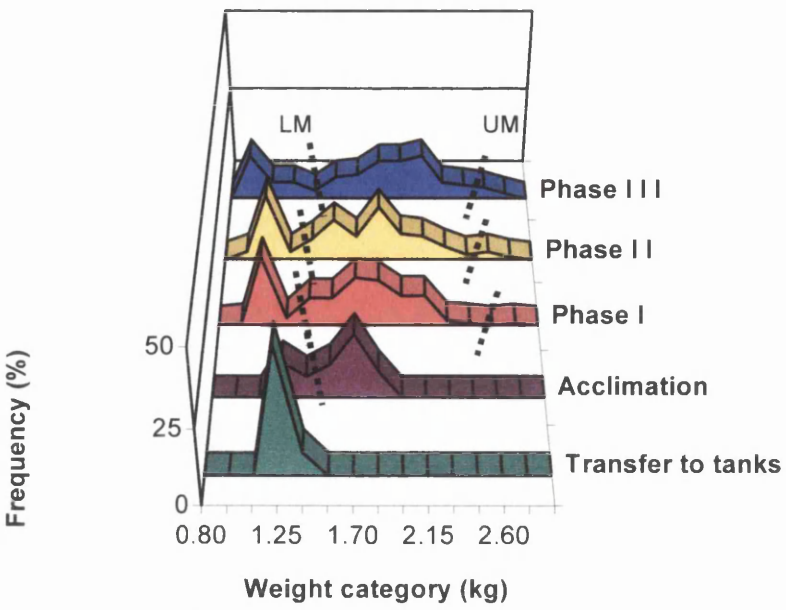


Figure 2.22 The relationship between feeding regime and CV for all fish from each tank at the start, 13 weeks, 21 weeks and 29 weeks.

Table 2.8 Summary of results from regressions on the relationship between feeding regime and weight variation, for the whole population (CVnp) and the main mode of each population (CV).

End of phase	CV				CV_{np}			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
I	0.493	1,8	0.058	*0.502*	6.108	1,8	0.433	*0.039*
I I	0.000	1,8	0.000	*0.992*	5.845	1,8	0.442	*0.042*
I I I	0.041	1,7	0.006	*0.846*	3.147	1,7	0.310	*0.119*

a) Low feeding regime



b) High feeding regime

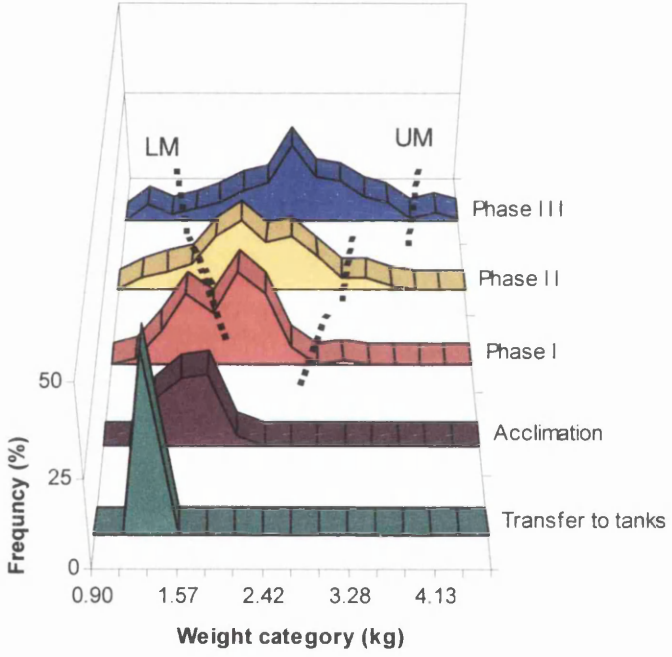


Figure 2.23 Weight variation of populations throughout the study, examples of populations fed on a) low feeding regimes and b) high feeding regimes. Dashed lines indicate cut-off weights of lower and upper modes. To facilitate comparison of phases I to III, transfer to tanks data was divided by three and acclimation data was divided by two.

Modal structure

Distinct upper and lower modes of fish could be found in all tanks, as illustrated in Figure 2.23. A series of chi-square tests of association revealed that there was a significant effect of feeding regime (expressed as high or low) on the proportion of individuals in each of the three modes at the end of phase I and phase II (Figures 2.23 and 2.24 a & b; phase I: $\chi^2 = 9.533$, d.f. = 2, $P = 0.009$: phase II: $\chi^2 = 15.599$, d.f. = 2, $P < 0.001$). Examination of the chi-square tables revealed the following trends: populations fed at low feed levels had more individuals in the lower mode and fewer individuals in the upper mode. Conversely, populations fed on high feed levels had fewer individuals in the lower mode and a greater number of individuals in the upper mode than expected. There was no effect of feeding regime on the proportion of individuals in each mode at the end of phase III (Figure 2.24 c; $\chi^2 = 3.962$, d.f. = 2, $P = 0.138$).

Given that there was an effect of feeding regime on the proportion of fish in each of the three modes, chi-square tests were carried out separately to ascertain if there were any changes in the proportion of fish in each mode over time. The tests revealed that there was no difference in the proportion of fish in each of the modes over time for populations fed on low or high feeding regimes (low feeding regimes: $\chi^2 = 3.492$, d.f. = 4, $P = 0.414$; high feeding regimes: $\chi^2 = 4.798$, d.f. = 4, $P = 0.309$).

The effect of feeding regime on the number of fish moving out of the upper, lower and main modes was also investigated. The total number of fish that moved modes was combined within the high and low feeding regimes and the proportion moving and staying was compared between populations fed on low or high feeding regimes using a chi-square test. The test indicated that there was no effect of feeding regime on incidence of movement in either phase (phase II: $\chi^2 = 1.060$, d.f. = 2, $P = 0.303$; phase III: $\chi^2 = 0.518$, d.f. = 2 $P = 0.696$).

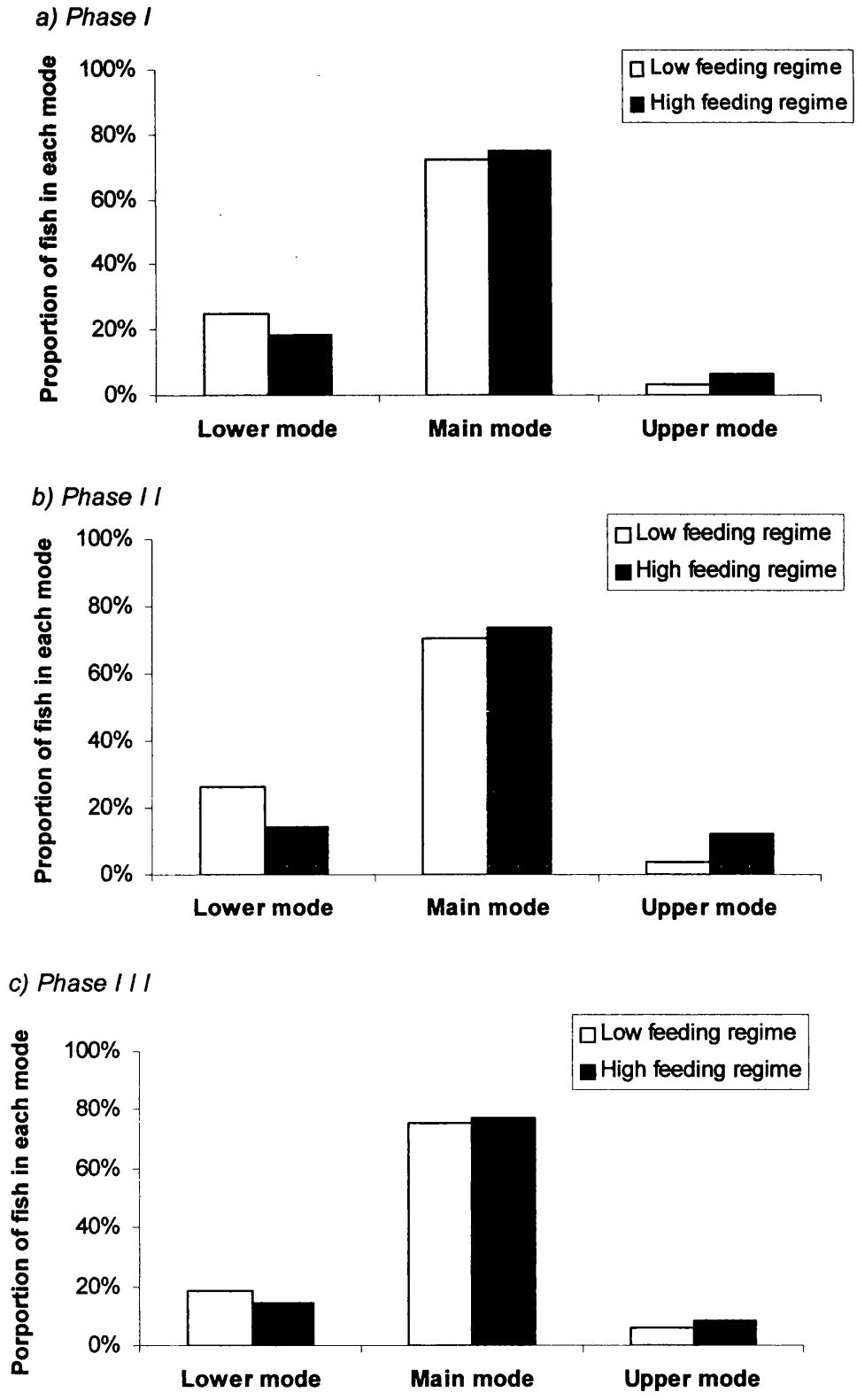


Figure 2.24 Proportion of fish in each mode at the end of a) phase I; b) phase II; and c) phase III.

Growth differences in high and low feeding regimes

In order to investigate whether there was a difference in growth within populations termed high (90% - 120%) and those termed low (30% - 50%), a repeated measures ANOVA with a *post-hoc* least significant difference (LSD) test on the effect of tank was carried out. For this test, growth was the within subject effect and tank was the between subjects effect (as tank data were normally distributed, whereas combined feeding regime data were not). This illustrated that there was an effect of tank on growth rate ($F_{8,335}$ = 17.892, P < 0.001). In addition, the LSD *post-hoc* test revealed that there was no difference in the growth rates of individuals grown on feeding regimes termed low, nor was there a difference in the growth rates of fish grown on feeding regimes termed high. However, there was a significant difference between the growth rates of fish from low and high feeding regimes.

As expected, there was a significant positive correlation between feeding regime and growth rate; populations fed on higher feeding regimes had higher growth rates (Figure 2.25; phase I: $F_{1,479}$ = 64.340, R-Sq = 0.118, P < 0.001; phase II: $F_{1,455}$ = 53.642, R-Sq = 0.105, P < 0.001; phase III: $F_{1,381}$ = 123.299, R-Sq = 0.244, P < 0.001). The slope of the relationship between feeding regime and growth at each sampling was compared to ascertain if the difference in growth of populations fed on high and low feeding regimes increased over time. The relationship between feeding regime and growth rate was steeper in phase III than phases I and II, (Figure 2.25; regression slope: phase I = 0.100; phase II = 0.108; phase III = 0.0188), indicating that the difference between the growth rates of low and high feeding regimes increased over time.

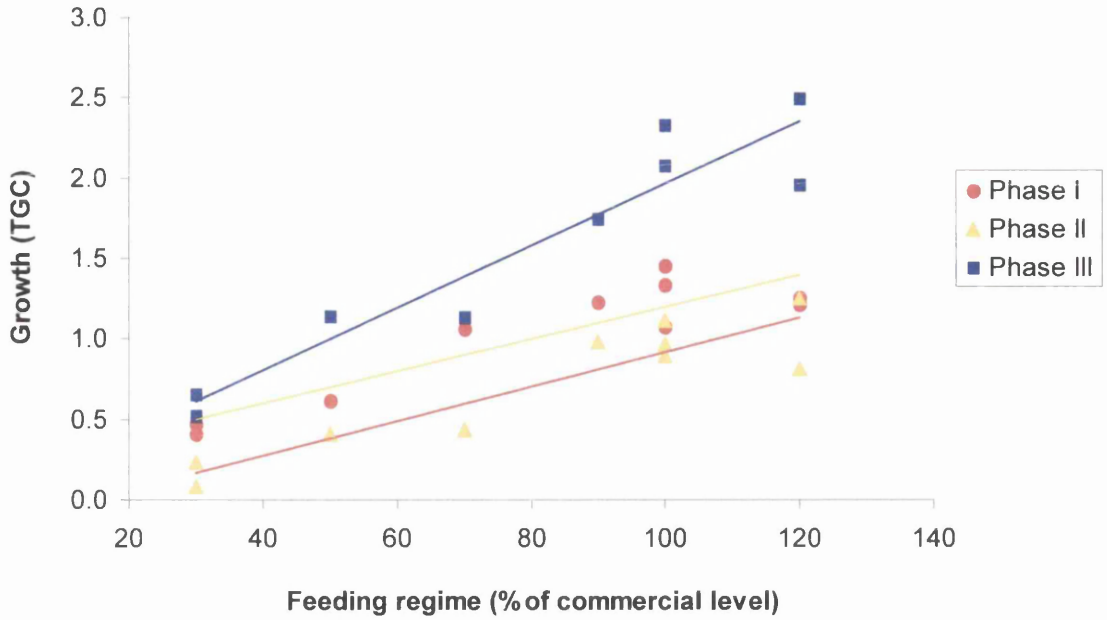


Figure 2.25 The relationship between feeding regime and growth during phases I, II and III.

Variation in growth

A significant negative relationship between feeding regime and variation in growth (CV) was observed in all phases. Fish from lower feeding regimes tended to exhibit a greater variation of growth rates (Figure 2.26; phase I: $F_{1,8} = 31.365$, R-Sq = 0.797, $P = 0.001$; phase II: $F_{1,8} = 35.690$, R-Sq = 0.817, $P < 0.001$; phase III: $F_{1,7} = 25.912$, R-Sq = 0.787, $P = 0.001$).

In addition, Wilcoxon signed ranks tests were performed to determine if there was an overall difference in the growth variation between each phase. The tests indicated that there was a significant difference in the growth variation found in each phase (Figure 2.26; Wilcoxon signed ranks test: phase I and phase II, $Z = -2.803$, $P = 0.005$; phase II and phase III: $Z = -2.666$, $P = 0.008$; phase I and phase III: $Z = -2.429$, $P = 0.015$). The greatest variation was found in phase II and the smallest in phase III. In conclusion, the greatest variation in growth occurred in populations fed on the lowest rations, where growth rates were lowest.

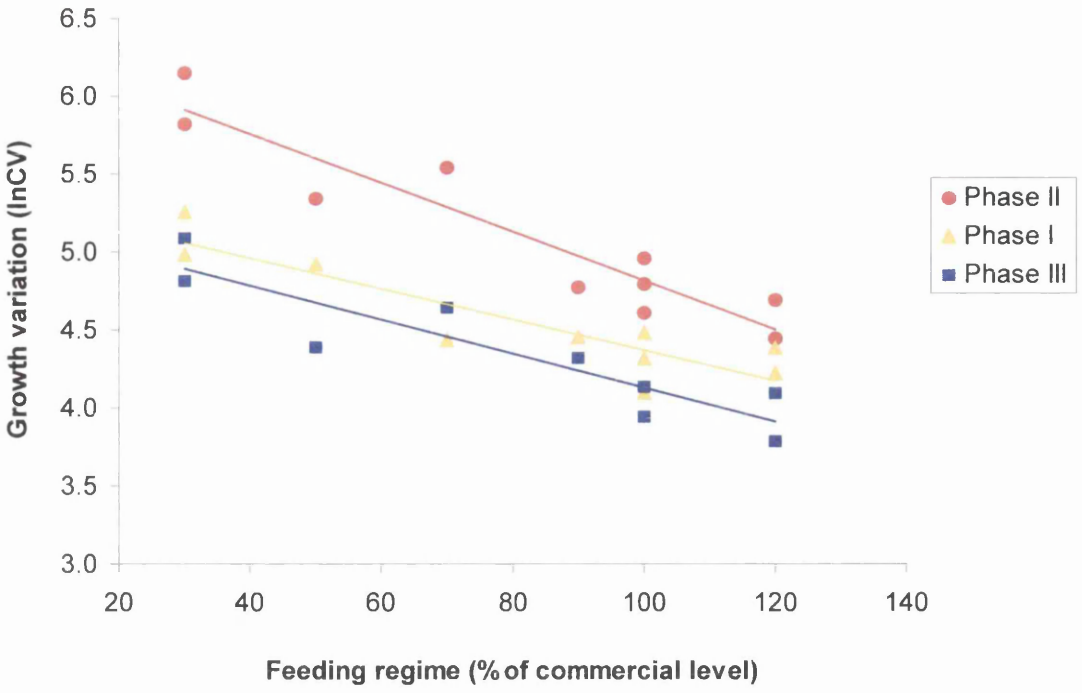


Figure 2.26 The relationship between feeding regime and growth variation during phases I, II and III.

2. 3. 3. 3 Condition

There was a significant positive relationship between feeding regime and condition at the end of phases I, II and III; fish grown under higher feeding regimes tended to have a higher condition score (Figure 2.27; phase I: $F_{1,1142}$ = 24.965, R-Sq = 0.021, P < 0.001; phase II: $F_{1,1037}$ = 49.136, R-Sq = 0.045, P < 0.001; phase III: $F_{1,877}$ = 114.051, R-Sq = 0.115, P < 0.001). In addition, there was a negative relationship between feeding regime and variation in condition (ln transformed CV) at the end of phase I, but there was a positive relationship at the end of phase II (Figure 2.28; phase I: $F_{1,8}$ = 6.372, R-Sq = 0.443, P = 0.036; phase II : $F_{1,8}$ = 8.313, R-Sq = 0.510, P = 0.020). Furthermore, there was no relationship after phase III ($F_{1,7}$ = 3.285, R-Sq = 0.319, P = 0.113). Closer inspection of Figure 2.28 suggests that the variation in condition decreased in all populations between phase I and phase II, but to a much greater degree in populations fed on lower feeding regimes. During phase III, the variation had

decreased relatively more in populations fed on high feeding regimes resulting in the absence of a relationship between feeding regime and variation in condition.

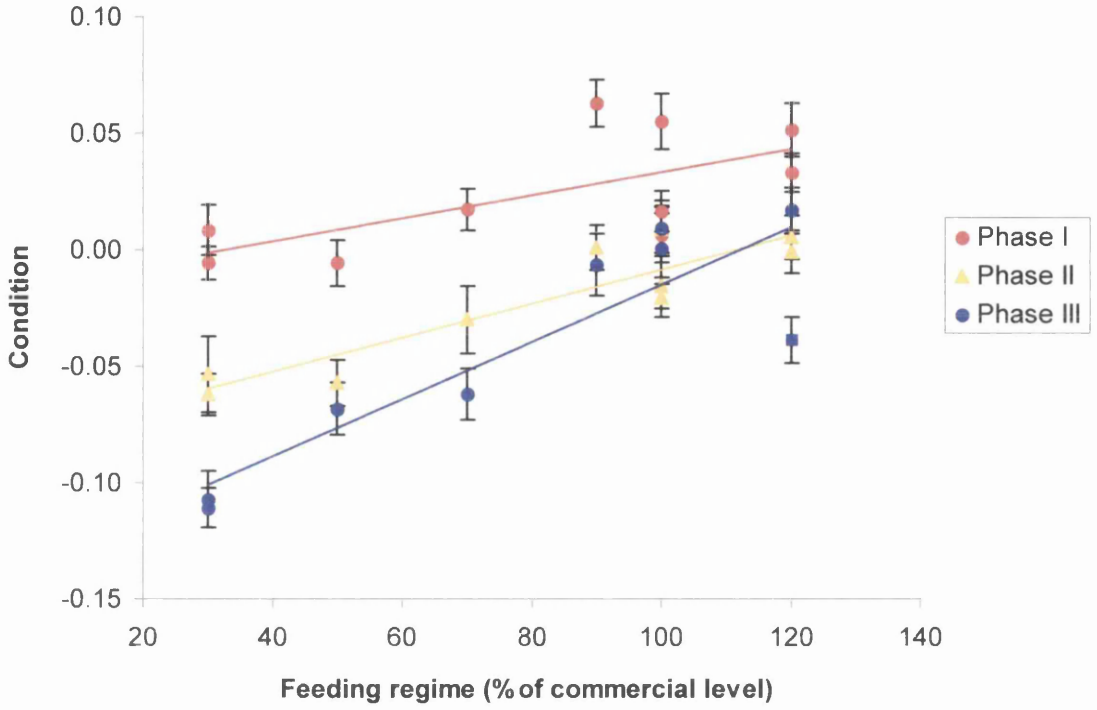


Figure 2.27 The relationship between feeding regime and condition at the end of phases I, II and III.

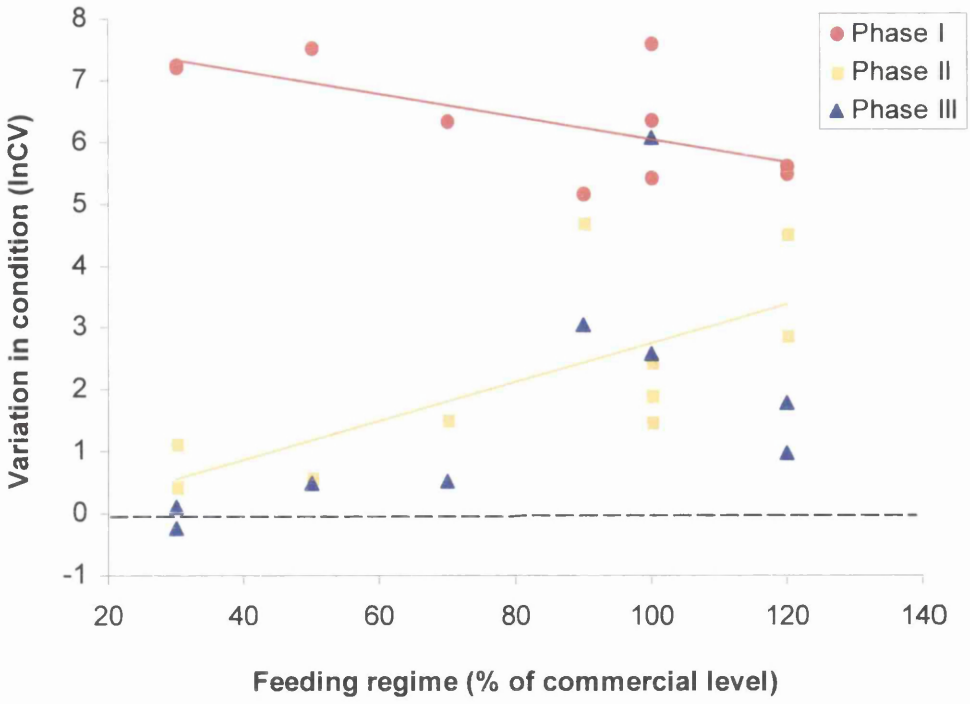


Figure 2.28 The relationship between feeding regime and variation in condition for phases I, II and III.

2. 3. 3. 4 Estimation of food intake

As individuals grown on low feeding regimes received less food than those on high feeding regimes, one might expect individuals from high feeding regimes to have eaten a greater amount of feed. However, this was not the case at the end of phases I or II, where the relationship between feeding regime and amount eaten (expressed as a percentage of an individual's bodyweight) was not significant (phase I: $F_{1,337} = 0.529$, $R^2 = 0.002$, $P = 0.468$; phase II: $F_{1,319} = 1.782$, $R^2 = 0.002$, $P = 0.183$). This was probably due to the high proportion of non-feeding fish during these samplings; 72% of fish did not feed at the end of phase I, and 52% of fish did not feed at the end of phase II. In contrast, at the end of phase III, when feeding was increased from 4 hours to 6 hours (resulting in 40% of fish feeding), there was a significant positive relationship between feeding regime and amount eaten (Figure 2.29; $F_{1,251} = 12.181$, $R^2 = 0.042$, $P < 0.001$).

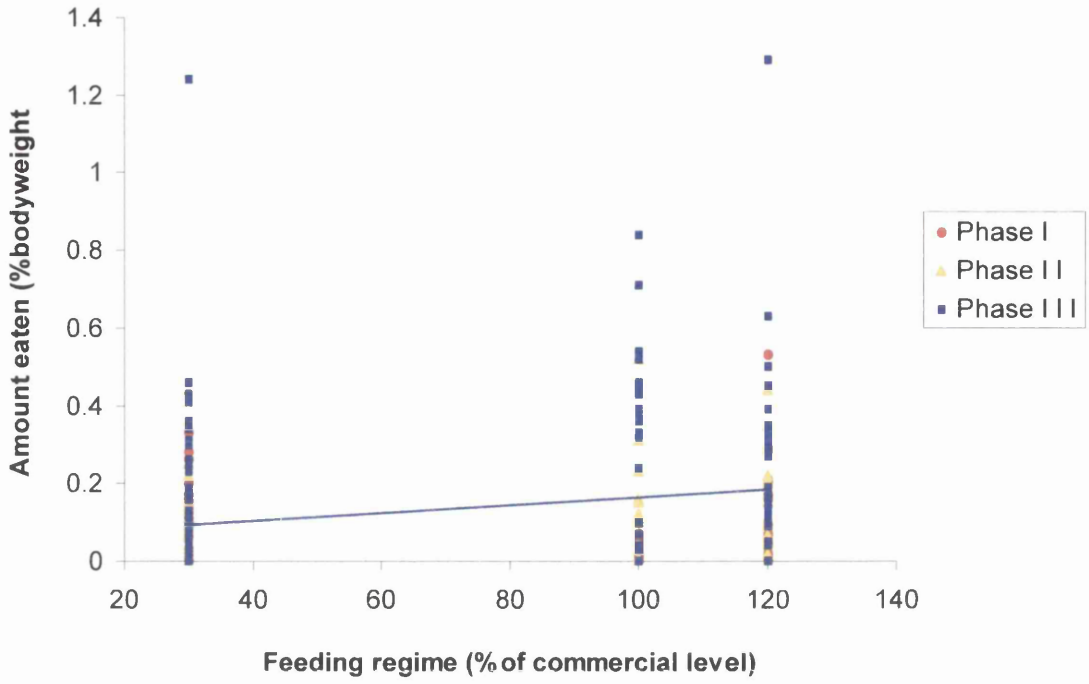


Figure 2.29 Relationship between feeding regime and amount eaten at the end of phase I, II and III.

There was no correlation between the amount eaten by individuals at the end of phase I and the amount eaten at the end of phase II for fish from high or low feeding regimes (high feeding regimes: $F_{1,93} = 2.432$, $R^2 = 0.015$, $P = 0.122$; low feeding regimes: $F_{1,70} = 1.177$, $R^2 = 0.017$, $P = 0.282$). There was also no relationship between the amount eaten by individuals at the end of phase II and the amount eaten at the end of phase III (high feeding regimes: $F_{1,93} = 0.033$, $R^2 = 0.000$, $P = 0.857$; low feeding regimes: $F_{1,70} = 0.062$, $R^2 = 0.001$, $P = 0.804$).

The relationship between total amount eaten and growth over the whole study was investigated separately for individuals grown on high and low feeding regimes at the end of each phase. This revealed that there was a significant positive relationship between amount eaten and growth in low feeding regimes at the end of phases I and II; individuals that ate more also grew more (Figure 2.30 a & b; Table 2.9). There was no

relationship between amount eaten and growth of individuals exposed to low feeding regimes at the end of phase III (Figure 2.30 c; Table 2.9). In populations grown on high feeding regimes there was no relationship between amount eaten and growth rate at the end of phases I and III (Figure 2.30 a & c; Table 2.9). However, at the end of phase II there was a significant positive relationship; larger fish tended to eat less (Figure 2.30 b; Table 2.9).

Table 2.9 Summary of results from regressions of amount eaten (% bodyweight) against growth rate (TGC) for fish fed on low and high feeding regimes at the end of phases I, II and III.

End of phase	Low feeding regime				High feeding regime			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
I	14.012	1,85	0.129	*< 0.001*	0.026	1,202	0.000	*0.873*
II	16.349	1,86	0.160	*< 0.001*	12.608	1,218	0.055	*< 0.001*
III	0.114	1,88	0.001	*0.736*	2.225	1,147	0.015	*0.138*

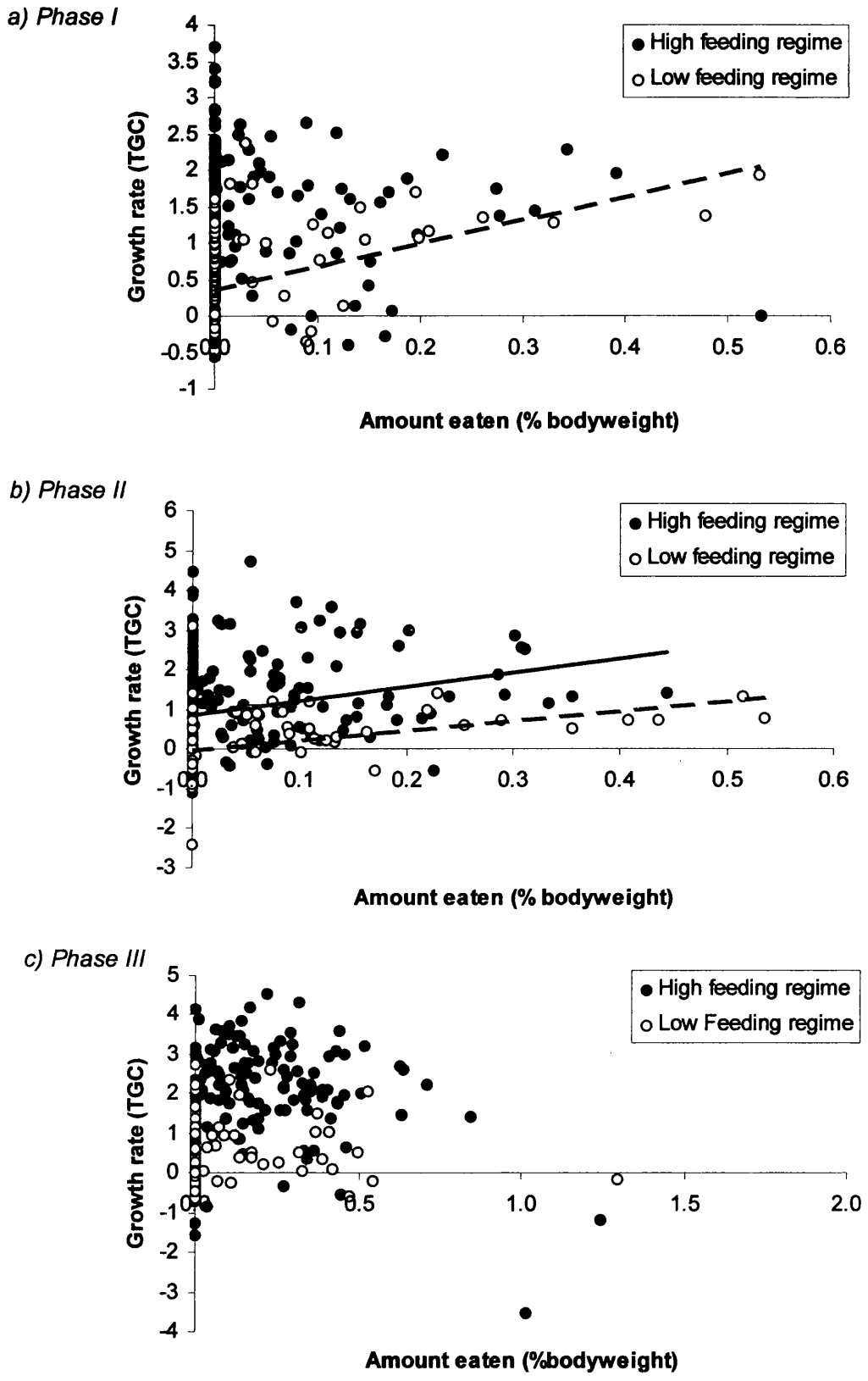
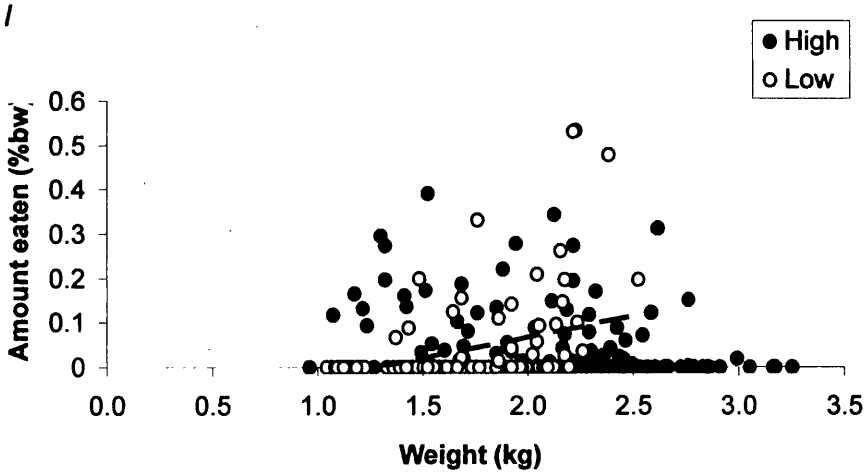


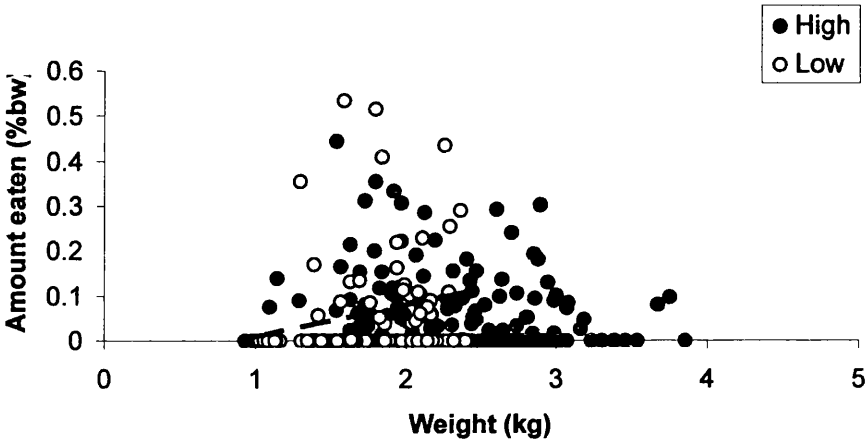
Figure 2.30 Relationship between growth and amount eaten in fish exposed to low (open circles and dashed lines) and high (filled circles and continuous lines) feeding regimes during a) phase I, b) phase II and c) phase III.

The relationship between fish weight and amount eaten was investigated separately for individuals grown on low and high feeding regimes. At the end of phase I and phase II, a positive relationship was revealed between weight and amount eaten in individuals from low feeding regimes (Figure 2.31 a - b; phase I: $F_{1,104} = 16.280$, $R^2 = 0.135$, $P < 0.001$; phase II: $F_{1,92} = 6.111$, $R^2 = 0.052$, $P = 0.015$). However there was no relationship between weight and amount eaten in individuals from high feeding regimes at the end of phases I and II (Figure 2.31 c; phase I: $F_{1,231} = 0.940$, $R^2 = 0.004$, $P = 0.333$; phase II: $F_{1,225} = 0.025$, $R^2 = 0.000$, $P = 0.871$). In contrast, there was a significant negative relationship between size and amount eaten in individuals from high feeding regimes at the end of phase III ($F_{1,251} = 12.181$, $R^2 = 0.042$, $P = 0.001$), but there was no such relationship found in fish from low feeding regimes at the end of phase III ($F_{1,93} = 0.915$, $R^2 = 0.010$, $P = 0.341$).

a) Phase I



b) Phase II



c) Phase III

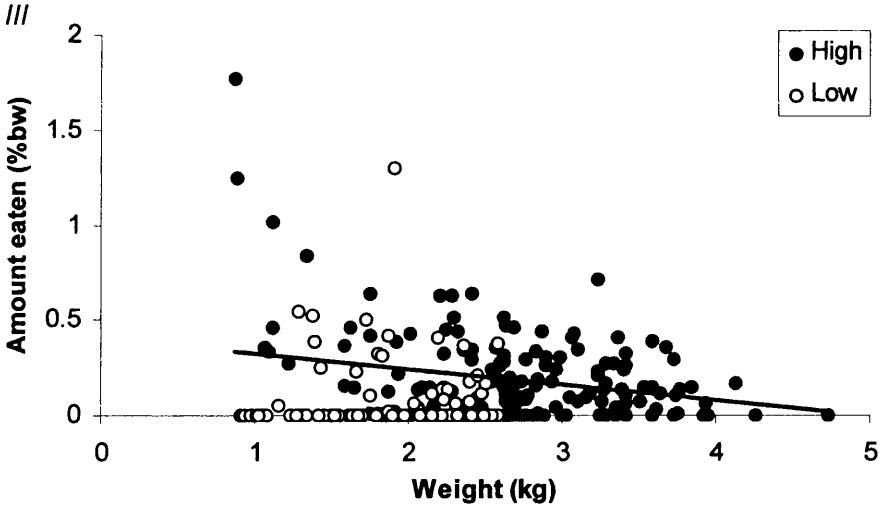


Figure 2.31 Relationship between weight and amount eaten in fish exposed to low (open circles and dashed lines) and high (filled circles and continuous lines) feeding regimes during a) phase I, b) phase II and c) phase III.

The variation in food intake (CV_i; see methods) over the whole experiment was then calculated for each individual. The variation of individuals from low and high feeding regimes was compared using a Mann-Whitney U test. This revealed that there was no difference in the variation in individual food intake in low and high feeding regimes (U = 1778.00, Z = -0.145, P = 0.885). The relationship between the average share of the meal that an individual obtained and the variation in feed intake of that individual was then examined by plotting regressions of CV_i against average share of the meal. There was no relationship between mean share of the meal and variation in amount eaten in fish from low feeding regimes ($F_{1,42}$ = 1.804, R^2 = 0.041, P = 0.186), but fish from high feeding regimes that had a smaller mean share of the meal tended to have a higher variation in the amount that they ate ($F_{1,80}$ = 31.399, R^2 = 0.282, P < 0.001).

However, this result was strongly influenced by a large number of individuals that were only observed to have eaten during one sampling (54% of fish in low feeding regimes and 53% of fish in high feeding regimes). The Cv_i of all of these fish, regardless of the amount that they ate was 173. Removal of these individuals from the analysis also removed the relationship between mean share of meal and variation in amount eaten in fish from high feeding regimes ($F_{1,36}$ = 1.155, R^2 = 0.031, P = 0.290). When fish that were only observed to eat during one sampling were removed from the analysis of fish from low feeding regimes there was still no relationship between mean share of meal and variation in amount eaten ($F_{1,18}$ = 0.126, R^2 = 0.007, P = 0.727).

Thus, there was no relationship between amount eaten by individuals at one sampling and the amount eaten at the next, and the greatest feed intake was observed at the end of phase III. Therefore, in order to ascertain if the distribution of feed intake was more polarised in lower feeding regimes, individuals were ranked by the share of the total amount of food recorded in each tank (see methods) at the end of phase III. Figure 2.32 illustrates that there were no clear differences in the polarisation of the

amount eaten. The distribution of food was highly polarised in one of the 30% tanks (Figure 2.32 b), but this pattern was not found in the other 30% tank (Figure 2.32 a).

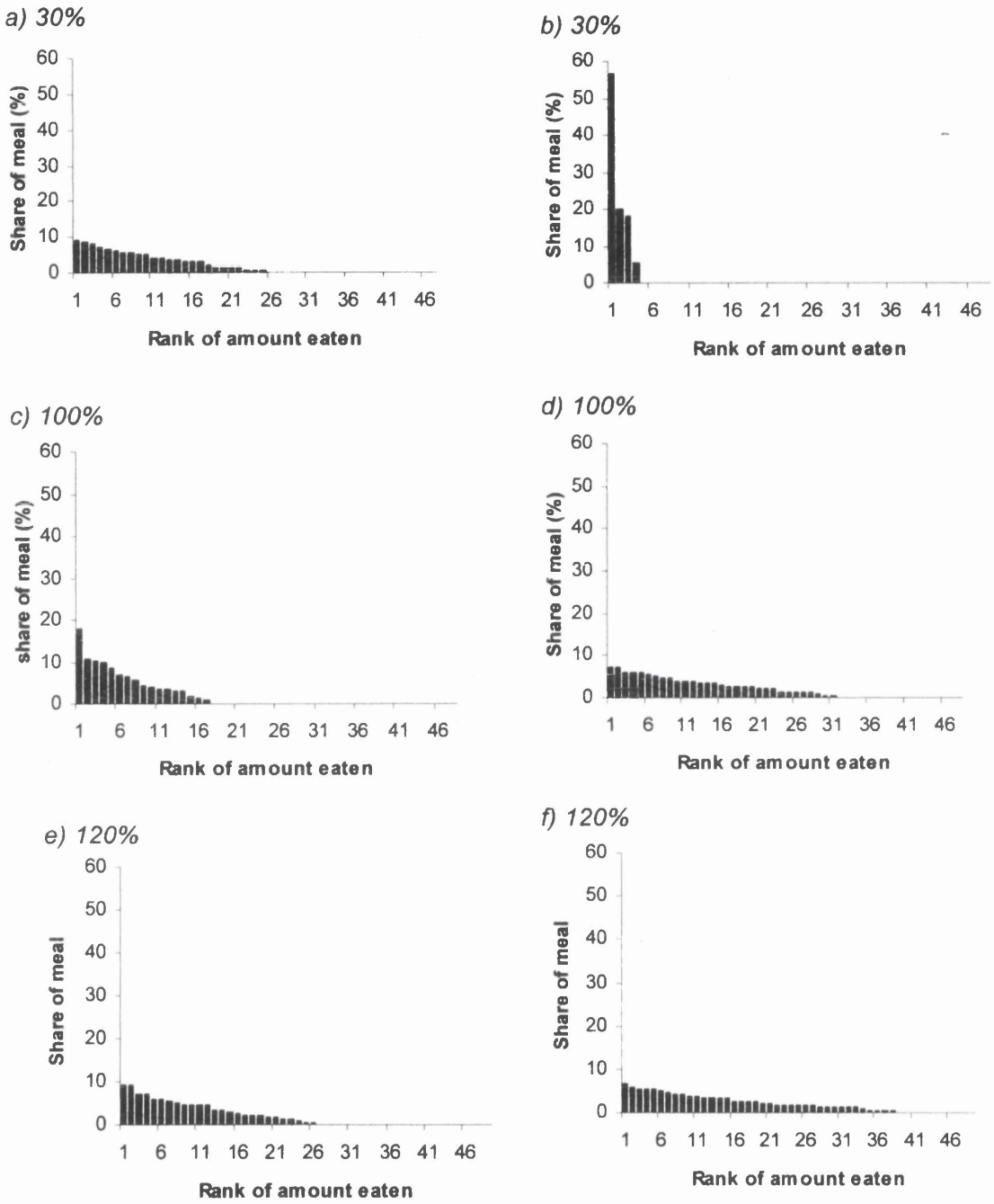


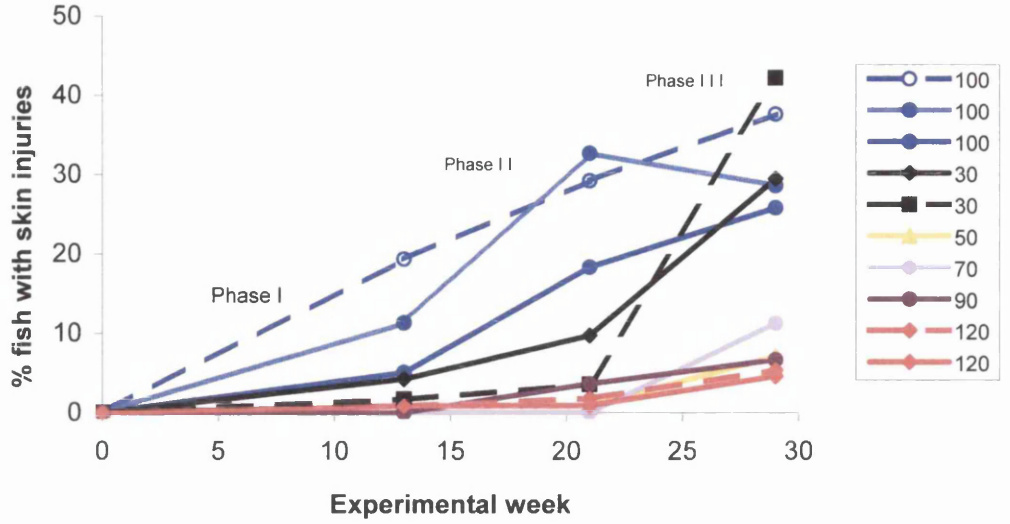
Figure 2.32 Share of meal (MSM) in fish exposed to feeding regimes of 30% (a & b), 100% (c & d) and 120% (e & f).

2. 3. 3. 5 Injuries

There were no clear relationships between the incidence of injury and feeding regime for either eye or skin injuries (Figure 2.33). As eye injuries were shown to influence

growth, the data were examined for evidence of an interaction between the effects of eye injury and competitive intensity (in the form of feeding regime) on growth. A two-way ANOVA was performed to look for an interaction between injury and tank. The test was only carried out on data from phase II and III, as the sample size of injuries per tank was too small in phase I. There was no interaction between injury and tank in either phase (two-way ANOVA, interaction effect; phase II: $F_{6,411} = 0.349$, $P = 0.910$; phase III: $F_{6,398} = 1.086$, $P = 0.370$). Therefore, competitive intensity did not influence the effect of eye injury on growth.

a) Skin injuries



b) Eye injuries

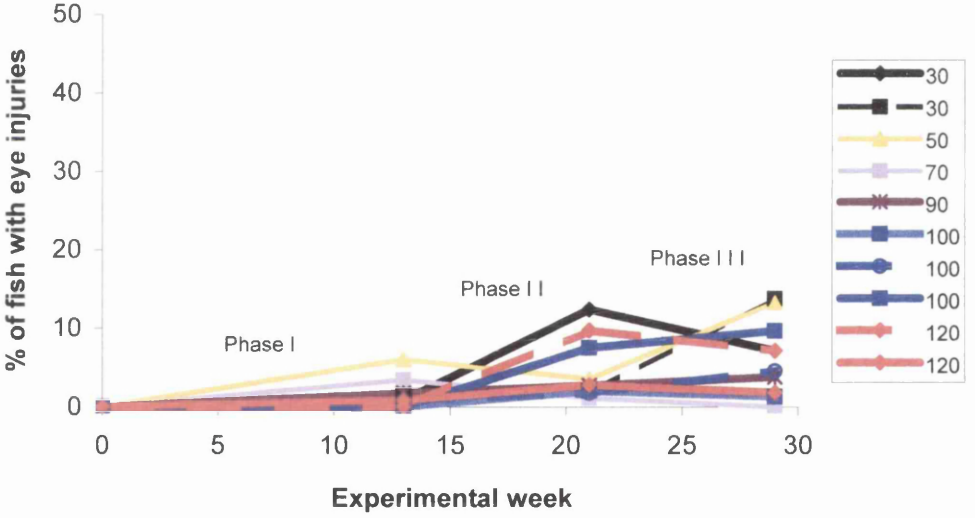


Figure 2.33 Proportion of fish with a) skin injuries and b) eye injuries at the end of phases I, II and III.

2. 4 DISCUSSION

This study monitored the development of size differentiation in populations of Atlantic salmon. Specifically, major differences in growth trajectory were identified and the individuals following these trajectories were characterised. The effect of ration on these processes was also investigated. The populations used for this study were initially very tightly graded, but they grew heterogeneously and therefore rapidly displayed a wide variation in size. After only four weeks, during which time the fish were acclimating to the experimental tanks, the weight variation within the populations increased to more than three times its initial value.

2. 4. 1 Evolution of size differentiation

As expected, weight and weight variation increased during the study. Differences in growth rates and the resulting weight variation have been observed in many species of fish (e.g. midas cichlids, Francis, 1988; Nile tilapia, Volpato *et al.*, 1989; coho salmon, Ryer & Olla, 1996; Atlantic salmon, Johansen & Jobling 1998; Johansen *et al.,* 2001). There was no relationship between weight variation and mean fish weight within each sampling of the described study, implying that variation increased over time and was not a direct result of increasing population weight. In addition, the increase in size variation was rapid initially, but changed by a much smaller degree thereafter. Irwin *et al.,* (2002) also found a rapid increase in variation in juvenile turbot and attributed it to increased competition during hierarchy formation. Furthermore, Irwin *et al* (2002) attributed the small degree of change after the initial increase to decreased competition due to the existence of the hierarchy. In small groups of fish, the frequency of escalated fights decreases over time (e.g. rainbow trout, Johnson & Åkeman, 1998; O'Connor *et al.*, 1999). If this were the case in the present study, the observed initial increase in variation would decrease as individuals developed strategies according to their newly established position in the hierarchy. For example Alanärä *et al.* (2001) found that subordinate brown trout were forced to feed at less favourable times by

dominant individuals when there was a high demand for food. In the current study, individuals lower in the feeding hierarchy may have learned to feed at different times or different locations from individuals higher in the hierarchy (see chapter 3).

Comparison of the gradient of the lower- and upper- mode cut off points over time revealed that the increase of the upper mode cut-off was steeper than that of the mean population weight while the increase of the lower mode cut-off was less steep. Therefore, this either indicates that size variation increases over time (which it does not conclusively) or alternatively, the number of lower and upper mode fish decreases over time. During the study, weight increased at a steeper gradient than the lower mode cut-off weight, suggesting that the number of lower mode fish would decrease until there were no longer any fish in this mode. In contrast the cut-off point of the upper mode had a slightly steeper gradient than mean weight, indicating that the weight of this mode increased faster than the weight of the population. As variation appears to be stable the upper mode cut-off may have become heavier than the largest fish, were the study continued.

2. 4. 2 General trends in growth trajectory

In order to investigate the differences in growth trajectory that caused the rapid increase in weight variation, the weight distribution was examined in detail. This revealed a bi-modal distribution at the start of the study. The presence of modes reflects an inequality in the structure of the population; individuals may choose, or be forced to follow different strategies, behaviourally or physiologically. Bimodal size distributions have been reported in the freshwater stage of wild Atlantic salmon, as a result of different strategies in the timing of sexual maturity (Thorpe *et al.*, 1982) and smolt migration (Heggenes & Metcalfe, 1991). In addition, Storebakken and Austreng (1987a) documented bimodality in (Atlantic salmon) fingerlings in commercial production conditions.

In commercial farming of salmon, bimodailty is sometimes observed in populations following smolt transfer as some individuals fail or are slow to adjust to living in sea-water. These individuals are often removed, but can catch up (D. Mitchell, pers com). There have been very few incidences where bi-modality has been reported after successful transfer to sea-water However, V. Crampton (pers com) observed lower modes in the size distribution of rainbow trout reared in experimental cages (5m x 5m x 5m) and there is circumstantial evidence to suggest that modes are present in commercial levels of production, but go undocumented (V. Crampton, pers com; S. Kadri pers com).

At the end of phase I, the weight distribution was tri-modal, and the number of individuals in the lower mode greatly decreased in size (by 50%). The population weight distribution was tri-modal for the remainder of the study and the majority of individuals were found in the same mode at the end of phase III as they were the end of phase I. The proportion of fish in these modes did not change, and the cut-off values increased with increasing mean weight, suggesting that the position of individuals in the distribution was unstable after 4 weeks, but was relatively stable by 13 weeks. These results support the idea that when new groups of fish are created a feeding hierarchy is formed and then stabilises.

2. 4. 3 Effect of season

Previous authors have noted that Atlantic salmon show an increase in growth rate in late spring (e.g. in wild juveniles, Gardiner & Geddes, 1980; and in commercial conditions, Forsberg, 1995; Mørkøre & Rørvik, 2001). In the described study, growth rate was lowest in phase II (March and April) and highest in phase III (May & June) suggesting that there was an effect of season on growth rates. The affect of season may have been delayed as the water was pumped from a depth of 30m; and may have caused delayed changes in temperature compared to other studies (e.g. see Forsberg, 1995; Mørkøre & Rørvik, 2001). Condition factor decreased rapidly initially, and to a

slower extent for the remainder of the study. The initial decrease in condition may have been triggered by a seasonal cue, as salmonids have been shown to become leaner over winter and fatter over summer (Kadri *et al.,* 1997; Sæther *et al.,* 1996). However, as the greatest decrease in condition occurred during phase I (mid winter), the initial decrease in condition may have been related to transfer from the sea cage to the tanks. Unfortunately, the condition of the fish when they were removed from the sea-cages is not known.

The seasonal cues that the subjects used may have been external or internal. Water temperature in the tank fluctuated and the small window would have allowed a small amount of daylight to enter the tank, especially on clear days. However, salmonids have been observed to display natural rhythms of growth when exposed to 12h light: 12h dark light regimes (Eriksson, 1978; Sæther *et al.,* 1996). Therefore, in salmonids, seasonal variation in growth is likely to be at least partly controlled by endogenous rhythms.

2. 4. 4 Possible causes of individual growth trajectories

Marked differences have previously been identified in groups of fish in terms of physiology e.g. metabolic rate and plasma ACTH level, (Metcalfe *et al.*, 1995; Cutts *et al.,* 1998; Balm *et al.,* 1994) and behaviour e.g. direction of the body tilting fright response and timing of feeding (Uchida *et al* 1993; Alanara *et al.,* 2001). These differences may represent different ways of competing for limited resources. In the natural environment, Metcalfe (1986) found that it was less energetically expensive for a subordinate juvenile Atlantic salmon to hide, than it was to actively compete for food. Such individual differences may be due to genetic variation (e.g. see Wang *et al.*, 1998; Volpato *et al.*, 1989). However, the subjects of the present study were successfully grown through the freshwater and sea-water-transfer stages of production and came from the mid-range of a population, so clearly had the capacity to perform well in production conditions.

It is unclear why there were so many individuals in the lower mode initially. The subjects were introduced to the tanks 4 weeks prior to the initial weight measurement. Therefore, it is possible that those in the lower mode took longer to acclimate to the tanks, giving them a competitive disadvantage. If this were the case, it is unlikely that they would remain in the lower mode for the remainder of the study, as many individuals did. Those that grew out of the lower mode were found to be larger in weight and in length than those that did not grow. There was no difference in the condition of fish that grew and those that did not grow. Rainbow trout and brown trout have been shown to grow in a cyclical fashion; alternating periods of synthesis with periods of apparent rest (Brown, 1946; Wagner & McKeown, 1985). If stepwise growth were equal throughout a population, the smallest individuals and / or those with the poorest condition would show growth spurts as they are more likely to have just undergone a period of apparent rest. However, the largest fish in this study moved up the weight distribution, implying that movement from the lower mode to the main mode was due to stepwise growth, but rather to individual ability.

The observed modes may indicate different behavioural strategies, comparable to subordinate and dominant individuals. For example, in a study with self-feeders Alanärä *et al.* (1998) demonstrated that there were three classes of Arctic charr in groups of 8 fish; those that activated the feeder and competed aggressively; those that did not activate the feeder or compete but fed, and those that did not feed or compete. Huntingford and Garcia de Leaniz (1997) suggested that Atlantic salmon with particular behavioural profiles might perform better under certain conditions. Furthermore, Metcalfe, (1986) found that it was less energetically efficient for subordinate juvenile Atlantic salmon in a stream to compete for food, than it was for them to hide and avoid competition. In the present study, particular individuals may have grown optimally in different modes. Individuals could have evaluated their growth rates and changed modes because of this evaluation. For example, if competition was more intense in the

upper mode but the rewards were also greater, individuals that failed to obtain enough food may have benefited by opting for a less competitive, less energetically expensive but less rewarding strategy, by moving from the upper to the main mode. The individuals that moved down one mode were in fact the smallest and therefore those with the lowest growth rates.

Alternatively, movement from one mode to another could be linked to cyclical growth. Wagner and McKeown (1985) demonstrated the occurrence of cyclical growth in rainbow trout, but they also noted that at any one time 25% of the population were out of synchrony with the population cycle. The individuals that were asynchronous grew asynchronously for one or two weeks, but then rejoined the population cycle. In the described study, the majority of individuals in the lower and upper modes remained in these modes, growing differently from those in the main mode for the duration of the study.

Weight at the end of the study strongly correlated with start weight (after acclimation), and weight at the end of phase I (after 13 weeks). In addition, initial weight explained 50% of the total growth and 71% of growth in phase I and was the single most explanatory variable. This may in part have been influenced by the method of growth measurement; thermal growth coefficient (TGC) provides a temperature corrected increase in grams per day. In large fish, a small increase in percentage bodyweight will result in a larger increase than a similar increase in percentage bodyweight in small fish. However, within the current study, large fish growing faster would result in initial differences in size variation increasing over time.

The correlation between weight and growth combined individuals from all feeding regimes. One might expect the effect of feeding regime to disguise such a correlation. However, this result suggests that large fish grew well and remained large irrespective of feed levels. These fish may be socially dominant individuals (see chapter 4), or

alternatively, intrinsically good growers or competitors (see chapter 6). Many studies have documented a positive relationship between size and growth in relation to social hierarchies in salmonids (e.g. Metcalfe *et al.,* 1995; Olsen & Ringo, 1999 and see Huntingford *et al.*, 1990). If larger individuals were dominant individuals, fish that were small and did not grow well may have been socially suppressed (Jobling, 1985; Koebele, 1985; Abbott *et al.,* 1985; also see chapter 5).

In terms of growth, a lower mode was also present during the first phase, but not thereafter. Individuals that did not grow remained in the lower mode of the weight distribution, while those that did grow were found in the main weight distribution at the end of phase I. In a similar study on rainbow trout, V. Crampton (pers com) found evidence of a lower mode in growth throughout the whole study. The distribution of growth for the remainder of the described study was not perfectly normal, but the modes were not clear and consistent.

2. 4. 5 Feeding regime and variation

Fish that were exposed to high feeding regimes tended to be larger and grow more. There was a positive relationship between feeding regime and weight variation of the whole population at the end of phases I and II, but this relationship was not present at the end of phase III. As there was no relationship between feeding regime and the weight variation of the main mode at any point in the study, this suggests that the increase in variation (of the whole population) in low feeding regimes stems from the individuals in the upper and/or lower modes. As populations grown on low feeding regimes had more lower mode individuals, these fish are likely to have caused the initial increase in variation.

The initial differences in the proportion of fish in the lower and upper mode, between low and high feeding regimes, were no longer present at the end of phase III,

suggesting that similar processes were affecting the distribution of fish in the modes of each feeding regime by this point.

Initially there was an effect of feeding regime on the proportion of fish in each mode, but this effect was not present at the end of phase III. Although the effect was not significant, there was a trend for less fish to be found in the lower mode over time.

As expected, growth variation tended to be higher in lower feeding regimes. Many authors have documented this trend, but the mechanisms behind it are unclear and are likely to vary according to conditions and species (e.g. midas cichlids, Francis 1988; turbot, Imsland *et al.*, 1998; whitefish, Jobling *et al.*, 1999). The difference in growth between high and low feeding regimes was higher in phase III than phase I and II, indicating that the relative difference in competition between high and low feeding regimes increased in phase III. Therefore, the greater availability of feed in high feeding regimes may have allowed the fish in these regimes to increase their growth rate during phase III in response to environmental cues. Subjects in lower regimes would be prevented from doing so by the low feed levels.

Overall, variation in condition decreased over the course of the experiment. However, there was an initial increase; variation in condition was greater at the end of phase I than it was for the remainder of the study. As the observed initial decrease in condition coincided with an increase in length, it is possible that some of the initial variation in condition was caused by individuals that were shorter at the start of the study. Therefore, when these individuals increased in length this would decrease the variation in condition of the population.

Alternatively, the observed change in condition may have been seasonal. Kadri *et al.* (1996b) observed a similar decrease in condition in non-maturing Atlantic salmon in sea cages. In the described study temperature decreased most rapidly during phase I

and in the wild, there is likely to be less food available during this time. Therefore, the subjects may have been adhering to evolved seasonal patterns in growth. Furthermore, Nicieza and Metcalfe (1997) found that salmon may increase relatively more in skeletal growth than in body mass when resources are scarce.

2. 4. 6 Food intake and growth

Although fish that were recorded with ballotini in their stomachs at every sampling had higher growth rates than those that were never recorded with food in their stomachs, the low incidence of feeding observed at the end of phases I and II questions the viability of the feed intake data. A positive relationship between growth rate and feed intake is has been documented in salmonids by many authors (e.g. Kadri *et al.*, 1995, McCarthy *et al.,* 1992a). However, Abbott and Dill (1989) found that there was a difference in the growth of dominant and subordinate rainbow trout, in groups of two, although they were fed equal rations, indicating that fast and slow growing fish may differ in their metabolism. In the present study there was no relationship between total amount eaten and growth rate, but this is likely to have been confounded by differences in feeding regime.

Feeding regime clearly affected patterns of feed intake within the populations. However, the fact that there was no difference in the amount of food consumed by fish in high or low feeding regimes at the end of phase I and phase II questions the viability of the X-ray data. It suggests that the large number of fish found to have no food in their stomachs might have influenced the result.

The feed intake data did reveal that, in low feeding regimes, larger fish tended to eat more and that individuals that ate more grew more at the end of phases II and I. It is perhaps surprising that this result was not found at the end of phase III when there were more recordings of fish having eaten. This may be as a result of different categories of fish feeding at different times (see chapter 3). Kadri *et al.,* (1997) found

that smaller, subordinate post-smolt Atlantic salmon fed at first and last light. Therefore, if there was a greater polarisation in the categories of fish feeding during the first 4 hours of feed delivery then this would be more apparent in the data from the first two phases.

At the end of phase III the opposite trend was observed in high feeding regimes; smaller fish tended to eat more. Salmonids are likely to monitor their body condition with respect to an expected value for a given time of year (Nicieza & Metcalfe, 1999) and are known to increase growth rates during summer (Sæther *et al.*, 1996; Mørkøre & Rørvik, 2001). Therefore, these smaller fish may have had a greater motivation to feed. This motivation may have been suppressed in low feeding regimes due to intense competition (see chapter 3), but the decreased competition in high feeding regimes may have allowed these fish to grow.

In the described study, large individuals that were exposed to low feeding regimes had a greater feed intake than smaller individuals, during the first two samplings. In addition, as fish that moved up from one mode to another were larger than those that did not, this indicates that size is important in feed acquisition. There was no correlation between the amount of feed eaten from one sampling to the next; a relationship that is indicative of consistent feeding hierarchies (McCarthy *et al.*, 1992a; Winberg *et al.*, 1993; Jobling & Koskela, 1996). Therefore, it is unlikely that the same individuals were consistently acquiring the largest rations. There may be a small and transient group of individuals that obtain the greatest amount of food and grow fastest (e.g. see Alanärä & Brännäs, 1996; MacLean *et al.*, 2000), as hierarchies are less stable in large groups (Fenderson & Carpenter, 1971).

The feeding regimes used in this study varied in the length of feed delivery as opposed to the number of deliveries. This may have increased the likelihood of food monopolisation, especially in the low feeding regimes as low ration levels promote

pronounced feeding hierarchies (McCarthy *et al*, 1992b). In addition, the speed of water flow in the tank was relatively fast, and carried food round the tank, but the pattern in which the food was carried round the tank was similar in every meal resulting in low and predictable feed deliveries.

2. 4. 7 Effect of feeding regime on condition

Feeding regime was observed to have a positive effect on condition; individuals grown on high feeding regimes tended to have a higher condition. Variation in the condition of each population was also affected by feeding regime, but the nature of the effect differed. At the end of phase I there was a negative relationship between variation in condition and feeding regime, but at the end of phase III there was a positive relationship. Furthermore, there was no relationship at the end of phase II. This may be due to a difference in the speed of the processes (physiological or behavioural) that determine the structure of a population in different feeding regimes. Feeding hierarchies may be established faster in lower regimes as competition is more intense. Initially, lower regimes had a greater size variation than higher regimes, but at the end of phase III there was no difference. Therefore, higher regimes had higher growth rates but no increase in weight variation.

The index of condition used in this study is a sensitive index of variation as it takes weight and length into account. Furthermore, it was calculated from weight residuals of the relationship between ln (length) and ln (weight) resulting in small numbers that could be positive or negative. Because of this method of calculation, it was possible for the standard deviation of the condition of a population to be larger than the mean. This resulted in very large values of variation in condition and the magnification of small differences in variation. One would expect there to be a higher variation in condition of populations grown on low feeding regimes, but the increase in variation in high feeding regimes at the end of the study is perhaps surprising.

2. 4. 8 Incidence of injuries

Many of the injuries recorded during the described study were caused by the feed collectors, and abrasive bolts inside the tanks. However injuries do occur in aquaculture and it is therefore relevant to look at the trends in incidence of injury and also the trends in healing of the injuries. As there were no injuries at the start of the study, the increase in the incidence of both eye and skin injuries during the first phase may have been due to acclimation to the tanks. The subjects used in this study were previously reared in 15m x 15m x 15m sea cages and therefore injuries may have occurred as they adjusted to a much smaller tank. The high injury incidence during phase I may also be due to the establishment of a feeding hierarchy or of fish learning which parts of the tanks to avoid. The incidence of injury also increased in phase III, and this may have been due to increased demand for food due to higher growth rates.

Injuries to the subjects' eyes were found to affect growth (also see chapter 5), but skin injuries did not appear to have any effect on growth. This was not surprising, as eye injuries are more likely to directly hinder feeding. However, this result could have been influenced by the nature of the injury and the sampling method. As there were relatively few skin injuries all incidences of injury were combined. In addition, there was a wider range of the degree of skin injury sustained; skin injuries ranged from mild abrasion of the subjects' scales, to small sores where flesh could be seen. In contrast, there was very little difference in the degree of severity of eye injuries; there were no mild eye injuries. The combination of mild and more severe skin injuries may have masked the effects of skin injuries. Differences in performance of fish with fin damage and bite marks has been documented, for example Christiansen and Jobling (1990) found that juvenile Arctic charr that sustained bite marks (in groups of 50) grew less well. However, bite marks are an obvious result of direct aggression, whereas the origin of the injuries sustained during the described study (with the exception of those in tanks with feed collectors) is not clear.

Subjects with eye injuries at one sampling had an increased likelihood of having eye injuries at the following sampling. This could be a result of eye injuries taking longer or to heal or being more energetically costly to heal. In addition individuals with eye injuries may have had reduced levels of food intake due to difficulties in obtaining food. Alternatively, there may have been a subset of fish that were particularly prone to injury or were particularly poor at healing injuries. Previous studies have demonstrated an association between social status and injury incidence than dominant individuals (Abbott & Dill, 1989; Adams *et al.*, 1998; Moutou *et al.* 1998; MacLean *et al.*, 2000), but the direction of this association (whether subordinate or dominant individuals had the greatest injury incidence) was not conclusive. Furthermore, as individuals that are socially stressed heal slower than non-stressed individuals (Schreck, 1981), differences in stress levels of different sizes of fish (see chapter 4) may have influenced healing.

2. 4. 9 Effect of commercial over- and under- feeding

The data in populations fed on near-commercial rations were examined for differences in weight, length and condition in tanks that were overfed, underfed and fed on normal rations from a commercial perspective. However, These comparisons revealed no differences in the populations of fish fed on near-commercial feeding regimes. This result was surprising as the populations were exposed to different feeding regimes for a period of 29 weeks and the total difference in the amount of food presented to fish in the 120% feeding regimes would be much higher than the total amount presented to those in the 90% feeding regimes. Therefore, one would expect differences in growth, weight and condition. As there were no statistically significant differences between these tanks this suggests that although there were differences in the amount of food presented to the populations, there were no differences in the amount of food that the fish actually ate. If the feeding regimes were set at too high a level then fish that were fed 90% of the commercial level may have been fed to satiation. The feeding regimes were calculated from growth tables that predicted the growth of the fish each day and

adjusted the feed level accordingly. The predicted weights were close to the actual weights at the end of each phase of the study, indicating that growth was close to the predicted rate. However, the fish may have undergone periods of slow and fast growth during this time, resulting in relative over-and under-feeding

Alternatively, if feeding was strongly polarised then an increase in feed level may only result in an increase in the amount of food obtained by a few, dominant individuals. This would result in an increase in growth in these individuals, but not in the population as a whole.

2. 4. 10 Conclusions and implications

The described study tracked individually marked individuals in populations of initially tightly graded fish. During the study the variation and structure of these populations over time was investigated independently and with respect to feeding regime. Although this study was carried out on a much smaller scale than current aquaculture practice, the results can be used to indicate areas that warrant further investigation in larger groups of fish.

Currently, individuals in aquaculture are regarded as part of a group, and generally not as distinct individuals. As there may be up to 100,000 fish in one cage, it is logistically impossible to consider every single fish. Individual weight is often calculated from the mean of a subgroup of the population and the result of this calculation can determine feeding regimes and date of harvest. Timing of disease treatment is also based on the degree of infection in a small sample of the population. However, the results of this study show that small groups of individuals follow different growth trajectories. This highlights the importance of sampling many individuals in order to gain general information about the population. In addition, it may be beneficial for farmers to develop management practices that take different growth trajectories, into account, in particular those of poor growing fish enhancing the growth of each group.

Further information is needed on the groups of individuals with different growth trajectories to ascertain the mechanisms behind these differences. The following chapters detail the part that social interactions play in the establishment of these subgroups and the feeding behaviour and competitive ability of fish from the observed modes.

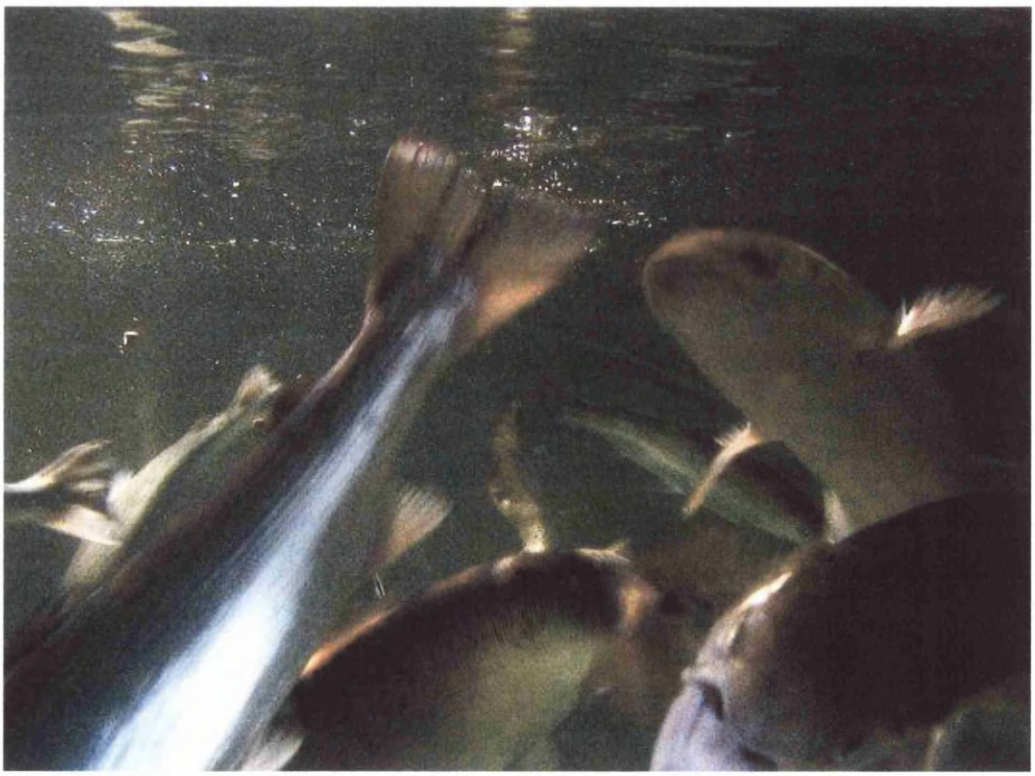
Size grading was initially thought to be beneficial for uniform growth, and in particular to encourage growth in small fish (e.g. common carp, Wohlfarth 1977; white sturgeon, Georgiadis *et al.*, 2000a; 2000b). However, it is now only carried out 2 or 3 times during the production cycle. The results of this study agree with an abundance of recent studies suggesting that size-grading does not enhance uniform growth (e.g. Arctic charr, Jobling & Wandsvik 1983; juvenile swordtails, Endemann *et al.*, 1997; Atlantic halibut, Stefánsson *et al.*, 2000). Current size grading removes the smallest individuals, but the rapid diversification observed in the present study suggests that the removal of these small fish will prompt others to move into their position in the weight distribution. Furthermore, as there is an indication that variation in weight may decrease after a period of time during which there is no grading, it may be beneficial never to grade the fish.

In aquaculture research, studies are often started with tightly graded groups of fish. However, the rapid increase in weight variation demonstrated in this study suggests that such tight grading is not necessary. In addition, the rapid diversification of weight may mask the true results of the variables being tested.

During phase I there was a sharp increase in weight variation. During the remainder of the study, the level of variation (of the whole populations) changed by a very relatively small amount. Rapid increases in size variation have been widely documented in salmonids (Johansen & Jobling, 1998) and in other animals; for example when piglets

are born, they are all very similar in size. During the period of lactation the weight variation increases dramatically and then levels off (Fraser *et al.*, 1995). Fast growth, and the resultant large body size can confer many advantages, for example in intraspecific competition (Harwood, 2001) and fecundity (e.g. snapping turtes, Iverson, 1997; butterfly, Kamata & Igarashi, 1995). However, there are also costs of fast growth e.g. reduced bone ossification in bluegill sunfish (Arendt *et al.*, 2001) and reduced resistance to starvation in speckled wood butterflies (Gotthard *et al.*, 1994). Therefore, the occurrence of differential growth within a population or group may be adaptive as different individuals may perform better in different conditions.

The following chapters investigate the mechanisms behind the observed variation in size in Atlantic salmon, focussing on individuals at the lower and upper end of the distribution.



Atlantic salmon feeding when exposed to a low ration

CHAPTER 3. BEHAVIOURAL STRATEGIES IN ATLANTIC SALMON IN LARGE GROUPS

3. 1 INTRODUCTION

In the natural environment, differences in competitive ability mediate access to restricted resources. Thus, the best competitors gain preferential access to these resources; for example, dominant piglets in a litter obtain the greatest amount of milk (Gill & Thompson, 1956). When the source of a food supply is spatially predictable, the best competitors are likely to occupy and / or defend the area containing the greatest amount of food (e.g. in aphids, Witham, 1979; and pigeons, Murton *et al.*, 1966).

Similarly, dominant juvenile salmonids occupy the most profitable territories within a stream (Keenleyside & Yamamoto, 1962; Fausch, 1984). In addition, individuals of differing competitive ability within a group may adopt alternative behavioural strategies resulting in different growth trajectories. For example, Metcalfe (1986) found that it was more energetically efficient for subordinate juvenile rainbow trout to hide than to actively compete for food. Similar patterns can result from temporal differences in resource availability; in brown trout, dominant individuals feed at dusk (when there are fewer predators) (Alanärä *et al.*, 2001). Alanärä *et al.*, (2001) also demonstrated that feeding times of some individuals (in a group of 5) changed in response to increased demand for food; the single most dominant fish fed at the preferred times, but the second ranking fish fed at other times. Therefore, the distribution, spatially and temporally, of particular categories within a species can change in response to increased competition.

In salmonid farming the fish that are reared today are relatively undomesticated compared to other farm animals. However, a combination of artificial selection for desired traits and inadvertent selection in hatcheries has resulted in differences in the behaviour of commercially reared compared to natural fish (reviewed by Ruzzante, 1994). Despite this, there are likely to be many behavioural traits that are retained from wild ancestors. For example, fish reared in tanks display rapid fright responses in response to overhead shadows and, particularly relevant in the present context, a tendency to fight over limited resources.

One consequence of aggressive competition amongst farmed fish is differences in growth rate. This has been documented in many species of fish (e.g. Arctic charr, Jobling, 1985; Atlantic salmon, Johansen & Jobling, 1998; greenback flounder, Carter *et al.*, 1996) and may be caused by poorer competitors being excluded from feeding by dominants (Jobling, 1985; Koebele, 1985). The presence of differential performance in large groups has also been demonstrated on a physiological level, Øverli *et al.* (1999a) found different physiological responses to the same stress response in subordinate and dominant Arctic charr. Further investigation of behavioural differences in fish of different sizes is necessary to determine the importance of social interactions in large groups of salmon at high densities.

In a previous study investigating the effects of feeding regimes on size variation, a tri-modal weight distribution was observed in all tanks (Chapter 2). The middle (main) mode contained the majority of subjects, and a small proportion (21% on average) made up the lower mode. The upper mode contained a smaller proportion of fish (6%), so upper mode fish were combined with large fish from the main mode to allow comparison of lower mode, small and large fish. In the present study, the feeding behaviour and tank position of lower mode fish was compared with large and small fish from the main mode. In addition, the effect of feeding intensity on behaviour was investigated with respect to the three size categories.

3. 1. 1 Aims

The aim of the described study was to investigate differences in the behaviour of fish from different sections of the size distribution (from the lower mode, and small and large fish from the main mode). Specifically, the following differences were investigated:

- The spatial distribution of fish of three size categories outwith feeding times
- The sequence of feeding attempts in fish of different size categories both with respect to time of day and time within a single feed delivery
- The effect of competitive intensity on the proportion of fish of different size categories feeding at different times of the day.

3. 2 METHODS

The study was conducted in two parts (see chapter 2). In part 1, size-graded groups of salmon were exposed to different feeding regimes in order to study the formation of size heterogeneity and the influence that feeding regime had upon this. The subjects were sampled three times, but otherwise left undisturbed and exposed to the same feeding intensities for 27 weeks. This provided long-established groups with different competitive intensities and a range of sizes. In part two, groups of fish from three size-categories were tagged and their behaviour was observed.

3. 2. 1 Experimental housing

During both part 1 and part 2, the subjects were housed in circular, 2m deep x 3m diameter experimental tanks. Seawater was supplied from a depth of 30m, providing an ambient water temperature. Water entered through a vertical pipe at the side at 210 $l.min^{-1}$ and exited in the centre, giving a circular flow. The oxygen level in the outlet ranged from 75 to 80% saturation and the light regime was 12h light and 12h dark for the duration of the experiment. Additionally, a small amount of natural light entered the tank through a 30cm diameter window on the tank wall, 1m from the tank base.

3. 2. 2 Part 1. Formation of stable groups

3. 2. 2. 1 Subjects

In November 1999, 1200 fish from the same stock, weighing 1.34 kg ± 3%, were selected from 15m x 15m x 15m deep sea cages. They were placed in 10 circular experimental tanks, giving a density of 15.16kg.m^{-3}. N.B. Commercial densities for Atlantic salmon are usually in the range of 12-15kg.m^{-3}. After four weeks of acclimation, 50% of the subjects were removed from each tank and fitted with VI tags (Northwest Marine Technology Ltd.) implanted in the post-ocular eyelid tissue (Niva, 1995).

3. 2. 2. 2 Feeding regime

In January 2000, following a further two weeks of acclimation, the subjects were exposed to experimental feeding regimes ranging from maintenance rations (30% of commercial feed levels) to overfeeding (120% of commercial feed levels). Commercial feed (EWOS Dynamic Red, size L) was used in all experimental tanks. The experimental rations were based on commercial feeding levels of 0.5% body weight per day. Behavioural observations were carried out on three tanks, each was fed one of the following rations: 70%, 90% or 120% of the commercial ration. They are referred to by their ration level in this chapter. Feed was delivered every 7.5mins between 07:00 and 19:00 from a pipe spanning the width of the tank. The duration of feed delivery was changed to provide the tanks with different rations, but the number of deliveries remained the same. The conditions for all 10 experimental tanks in part 1 are outlined in detail in chapter 2.

3. 2. 2. 3 Sampling, tagging and definition of size categories

The subjects were sampled 13, 21 and 29 weeks after tagging. During each sampling in part 1, individuals were anaesthetised (Aqui-S, New. Zealand), weighed, measured, and identified. In addition, during the sampling after 29 weeks (8th July 2000), tags for behavioural observation were fitted to 3 size categories of fish; small and large fish

from the main (and upper) mode and all lower mode fish (Figure 3.1). Fish termed as 'large' consisted of individuals from the main mode as in addition to those in the upper mode as the sample size in the upper mode was small (6% in the upper mode compared to 21% in the lower mode). The behavioural observation tags consisted of 1cm x 3cm of coloured waterproof paper and were attached to the subjects' dorsal fin using 3cm long Kimble clothing tags (UKSS, Manchester). Fish of different size categories were identified by different colours of waterproof paper; white paper was used for lower mode fish, green for small fish and red for large fish.

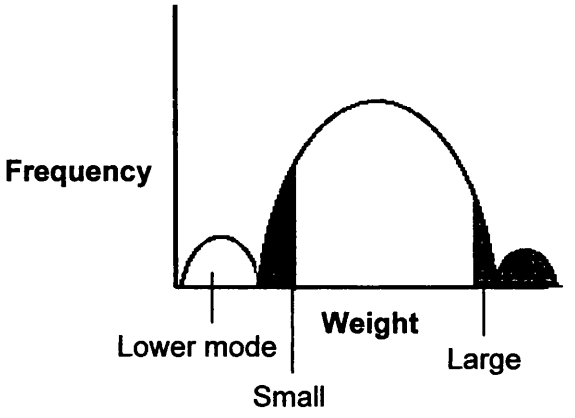


Figure 3.1 Weight frequency distribution graph showing the size-categories of tagged fish.

An estimate of the population weight distribution in each tank, calculated by initially sampling one third of the subjects, was used to determine the weight ranges for the three size categories during sampling. Therefore, a third of all fish in each tank (chosen randomly) were not tagged.

3. 2. 3 Part 2. Behavioural observations

3. 2. 3. 1 Conditions

During part 2, the median temperature was 8.1°C (range: 7.5 - 11.5°C) and the feeding regimes were continued from part I. The median weight of each population and the number of tagged fish in each category are summarised in Table 3.1. In the described study three tanks, in which the fish were exposed to different feeding regimes, were observed. The results from a previous study (chapter 2) indicated the occurrence of discrete subordinate-groups of fish in groups exposed to a range of regimes. The absence of replicates in the present study allowed a greater amount of information to be collected from populations exposed to slightly different, but comparable, feed levels.

Table 3.1 Summary of weight, proportion of individuals tagged and density in each tank

		Tank		
		70	90	120
Median weight (kg) (and interquartile range)		2.26 (0.72)	2.50 (0.98)	2.61 (0.78)
Population size		89	105	106
Density (kgm^{-3})		18.4	24.1	25.8
Number of tagged fish (and % of total population)	Lower mode	11 (12%)	17 (16%)	17 (16%)
	Small	5 (6%)	8 (8%)	20 (19%)
	Large	21 (24%)	12 (11%)	16 (15%)

3. 2. 3. 2 Collection of behavioural data

Behavioural observations started after two weeks of acclimation following size-tagging. Data were collected using video footage, recorded through a circular 30cm diameter window in the tank wall. The window was 1m from the base of the tank and provided a view of an area of 2m of the back wall of the tank (Figure 3.2). The tank wall was divided into three sections in order to quantify the vertical position of individuals. Two

lines of waterproof tape were applied at 50cm intervals during sampling, creating lower, middle and upper sections. Tanks were filmed in rotation for one day at a time over 18 days (6 days per tank). Filming took place between 07:00 and 19:00 hours, the hours of food presentation and tank illumination. The subjects were filmed for 6 x 30 min phases each day; at 07:00, 09:15, 11:30, 14:00, 16.15 and 18:30. The phases were termed 1 to 6, and each contained four feed deliveries.

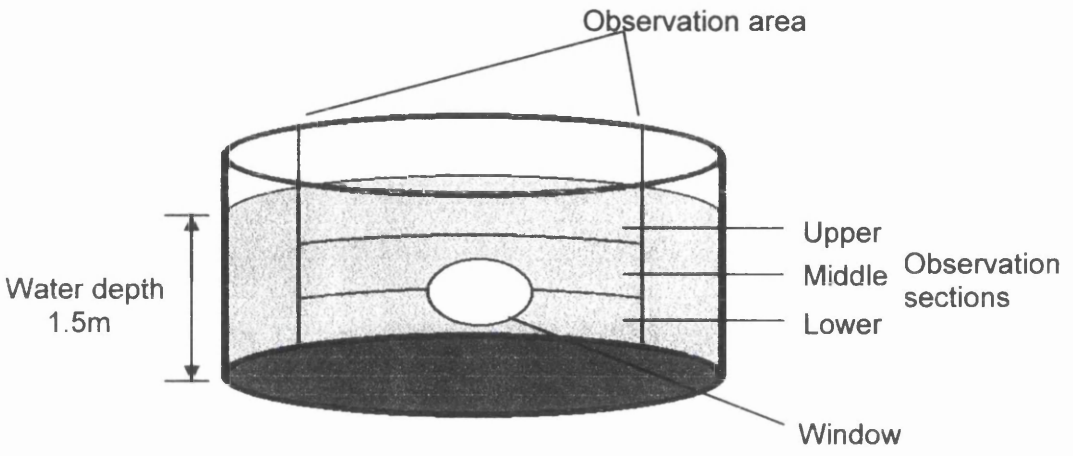


Figure 3.2 Schematic of observation tank layout.

3. 2. 3. 3 Tagging: tag loss and effects on behaviour

In order to investigate the effect of tagging on fish behaviour, and also the effects of feeding regime and fish size on tag loss, data were collected from the first 4 days of footage for each tank. The number of tagged and non-tagged fish in total and in each section was counted by freezing the picture on the screen 1 minute before feed delivery.

The effect of tagging on behaviour was investigated by comparing the proportion of tagged to non-tagged fish in relation to time of day and feeding regime. In addition, the spatial distribution of tagged and non-tagged individuals (outwith feed delivery) was

compared. Initially, data from each day of observation were combined for each tank to investigate differences in the proportion of tagged to non-tagged fish observed throughout the day (during the 6 time periods). A series of chi-square tests indicated that there was no difference, in any tank, in the proportion of tagged to non-tagged fish observed in the 6 time phases (70%: $\chi^2 = 1.742$, d.f. = 5, $P = 0.884$; 90%: $\chi^2 = 1.402$, d.f. = 5, $P = 0.924$; 120%: $\chi^2 = 1.035$, d.f. = 5, $P = 0.960$).

Data from the 6 time phases and each day of observation were therefore combined to test for differences in the proportion of tagged to non-tagged fish observed in each tank. A chi-square test of association revealed a greater proportion of tagged fish observed in the 70% tank ($\chi^2 = 24.035$, d.f. = 2, $P < 0.001$). This was likely to have resulted from increased numbers of tagged fish observed there on days 3 and 4 of filming in this tank. Lastly, the proportion of tagged and non-tagged fish in each vertical section of the tank was analysed. Data from each day of observation and each time phase were combined for each tank. In each tank, chi-square tests revealed that tagged fish were more likely to be observed at the bottom of the tank than non-tagged fish (Figure 3.3; 70%: $\chi^2 = 50.721$, d.f. = 2, $P < 0.001$; 90%: $\chi^2 = 82.417$, d.f. = 2, $P < 0.001$; 120%: $\chi^2 = 78.562$, d.f. = 2, $P < 0.001$).

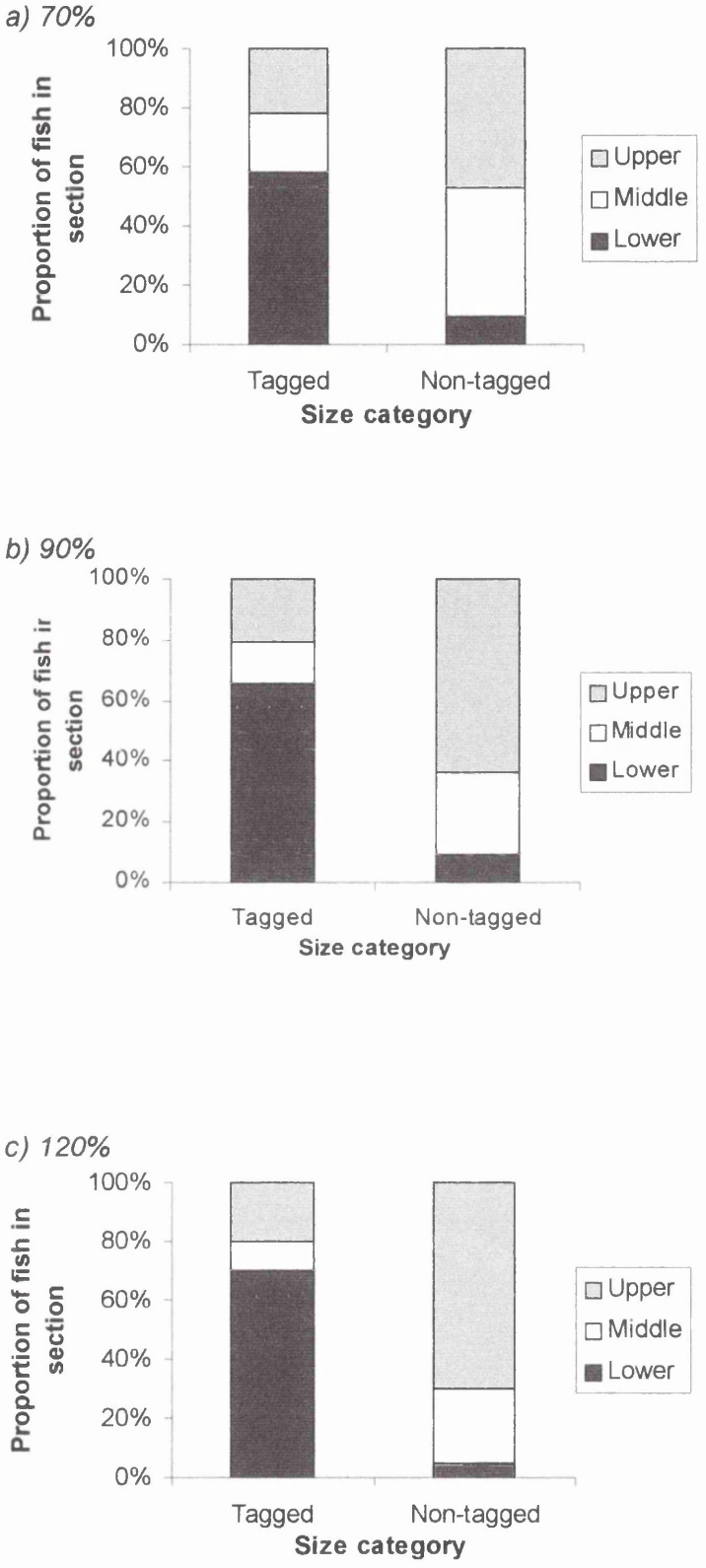


Figure 3.3 Proportion of tagged and non-tagged fish found in the upper middle and lower section in populations exposed to feeding regimes of a) 70%, b)90%, c) 120% of commercial rations.

There was a substantial degree of tag loss during the period of behavioural data collection; a tag loss of 62% was recorded 1 week after filming finished, when the fish were sampled. To ascertain if there was a difference in the proportion of tagged to non-tagged fish over time, chi-square tests were carried out on the number of tagged and non-tagged fish observed in each tank. Data from each feed delivery and each 30 min phase was combined for the first four days for each tank. These tests revealed that there was no difference in the proportion of tagged and non-tagged fish observed over time in the 90% and the 120% tanks (90%: $\chi^2 = 0.503$, d.f. = 3, $P = 0.918$; 120%: $\chi^2 = 3.251$, d.f. = 3, $P = 0.355$). However, there was a difference in the 70% tank; on days 3 and 4 there was a much greater proportion of tagged fish observed than non-tagged fish (Figure 3.4; $\chi^2 = 10.254$, d.f. = 3, $P = 0.017$). This effect was unlikely to be due to tag loss as the number of observed tagged fish increased. The extent of tag loss in fish in the different size categories was then compared using a chi-square test for each tank. The number of tags retained in relation to the number of tags lost in each size category was tested for each tank. There was no difference in tag loss of different size categories in the 70% tank ($\chi^2 = 5.76$, d.f. = 2, $P = 0.056$). However, there were clear differences in the 90% and 120% tanks; larger fish were least likely to loose tags (Figure 3.5; 90%: $\chi^2 = 6.937$, d.f. = 2, $P = 0.031$; 120% $\chi^2 = 9.72$, d.f. = 2, $P = 0.008$).

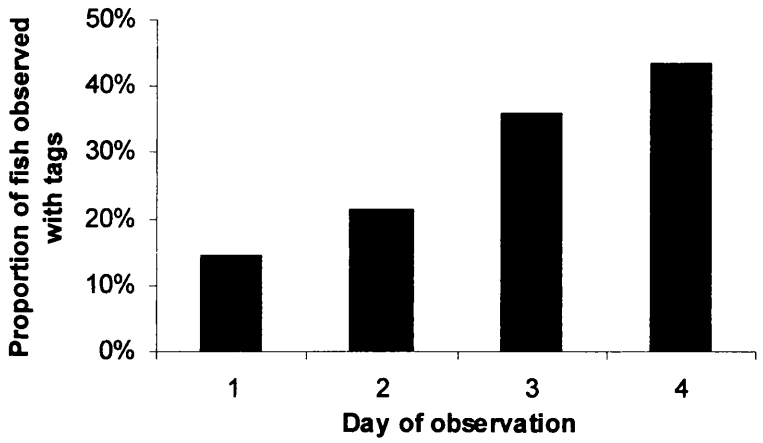


Figure 3.4 Proportion of fish observed that were tagged over four days of observation in the 70% tank.

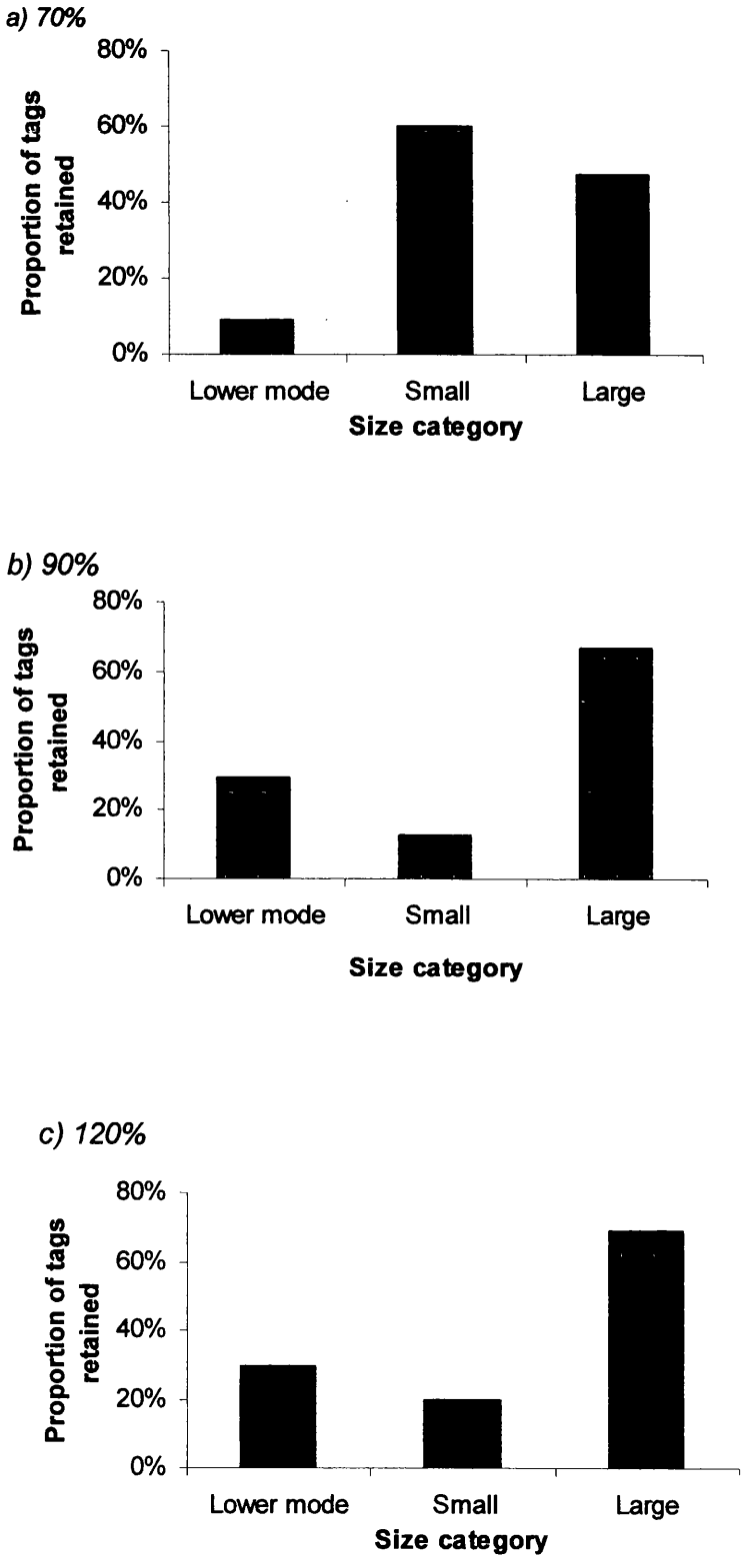


Figure 3.5 Proportion of tags retained by lower mode, small and large fish in populations exposed to feeding regimes of a) 70%, b) 90% and c) 120% of commercial rations.

These results suggest that the described method of behavioural tagging affected the behaviour of fish. Previous studies have shown that injuries gained during tagging can reduce the rank of an individual in a dominance hierarchy (Vascotto, 1970). Although there were no external injuries caused by tagging in this study, the observed differences in behaviour and differential tag loss have implications for interpretation of the behavioural data.

If tagging affected the behaviour of all size ranges of fish it may have resulted in behavioural observations of fish that were not representative of normal behaviour of any size of Atlantic salmon in large groups. Furthermore, if tagging affected different sizes of fish in different ways, this could disguise true differences in the behaviour of different sizes of fish. As fish in the three categories lost tags differentially, it is likely that tagging did affect fish of different sizes to a varying degree. Large fish had the smallest degree of tag loss; therefore, they may have been over-represented in the data compared to small and lower mode fish. As small and lower mode fish lost tags to a greater degree than large fish, differences between these size categories could be less distinct. The effect of tagging and tag loss on behaviour is further discussed with respect to the behavioural results in the discussion.

3. 2. 3. 4 Video analysis

For each day of video footage, the vertical position of tagged fish outwith feeding was recorded by freezing the video picture 1 minute before feed delivery. In addition, for each feed delivery within each section of the day, the number of fish of different size categories that fed was recorded. The sequence that fish of different size categories fed was also determined from each feed delivery. However, it was not possible to ascertain whether individuals actually ate food, so individuals that swam directly towards feed or the area of the tank where other subjects were feeding were defined as feeding. The sequence of feeding was calculated (in seconds) in relation to the duration of each feed delivery. The duration of feed deliveries were defined as the time

at which the first pellets hit the water surface until the time when pellets were no longer visible in the tank and there were no more feeding attempts.

3. 2. 3. 5 Data analysis

Initially, the vertical position in which fish of different size categories were found outwith feed delivery was investigated. All position data were combined for each tank and chi-square tests were carried out for each tank to compare the proportion of fish of each category in each vertical section of the tank. In order to determine if fish of different size categories fed at different times of the day, feeding data from all days were combined for each tank. The proportion of fish of each size category that fed during each time phase was then compared within each tank using chi-square tests.

The effect of ration on the proportion of fish of different size categories feeding was analysed by expressing the number of feeding individuals of a given category as a proportion of the total number of tagged fish in that category (adjusted total for tag loss, assuming a linear rate of tag loss). The number of tags of each category initially placed on the fish and the number of tags at the following sampling were plotted against the days of the study. This was used to calculate the adjusted total number of fish with each type of tag. Regressions of the proportion of fish of each size category feeding against feeding regime (70%, 90% and 120%) were then collated.

Data on the feeding sequence of different categories of fish within a food delivery were corrected for the duration of feed delivery. The sequence (time in seconds from the start of the feed delivery) at which an individual attempted to feed was divided by the duration of the feed delivery (as feeding regime was altered by changing the duration of the feed delivery). Data from each day was combined and the effects of size category and time of day and the interaction between these effects were examined using Scheirer-Ray-Hare (Dytham, 2000) tests for each tank.

No data was collected from 2 feed deliveries in the 70% tank and 90% tank or from 3 feed deliveries in the 120% tank due to condensation on the tank window. This resulted in data from a total of 142 feed deliveries from 6 time sections on each of 6 days of in the 70% and 90% tanks, and from 141 feed deliveries from 6 time sections on each of 6 days of in the 120% tank.

3. 3 RESULTS

3. 3. 1 Spatial distribution outwith feed delivery

In every tank, there was a difference in the vertical position of fish in the three size-categories; lower mode fish were more likely to be observed in the lower level of the tank and large fish were more likely to be observed in the upper level of the tank (Figure 3.6; 70%: $\chi^2 = 100.365$, d.f. = 4, $P < 0.001$; 90%: $\chi^2 = 45.753$, d.f. = 4, $P < 0.001$; 120%: $\chi^2 = 62.935$, d.f. = 4, $P < 0.001$). Furthermore, the distribution of small fish was similar to that of large fish rather than to fish from the lower mode (Figure 3.6).

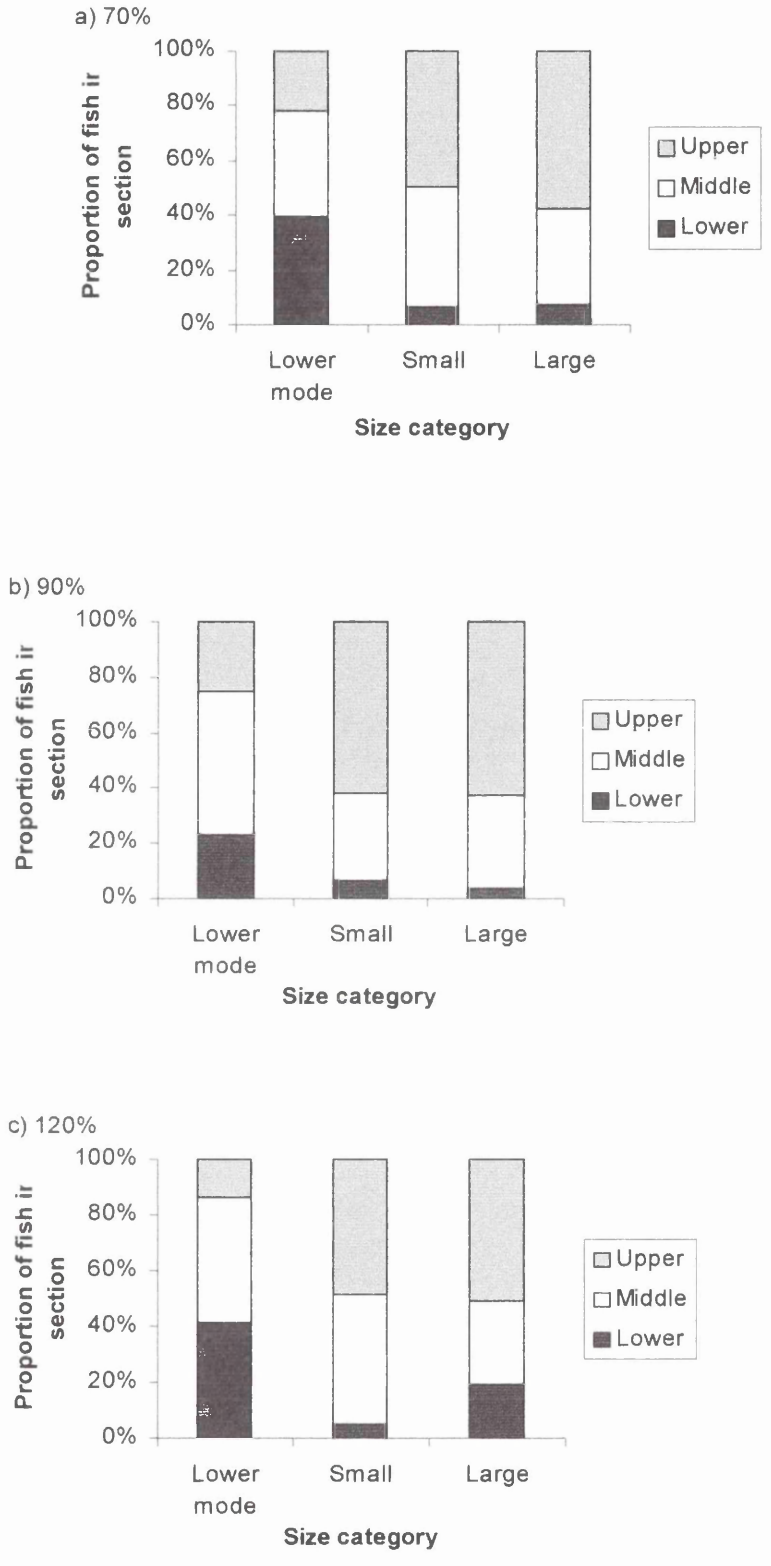
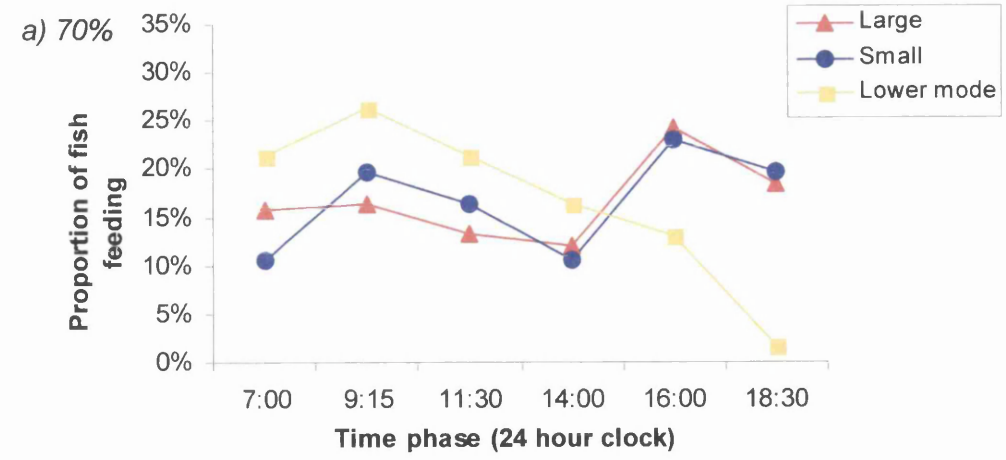
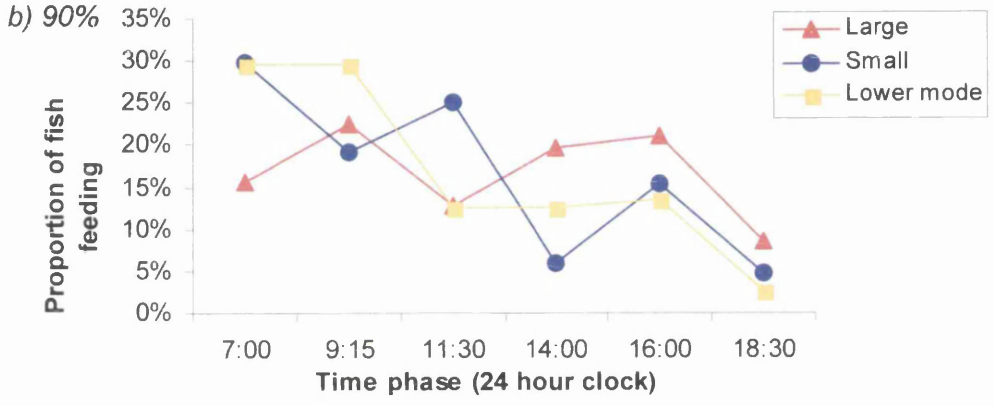


Figure 3.6 Proportion of lower mode, small and large fish observed in the lower, middle and upper sections in populations exposed to feeding regimes of a)70%, b) 90% and c)120% of commercial rations.

3. 3. 2 Feeding behaviour

Analysis of the time of day that fish of different size categories fed revealed a difference in the 70% and the 90% tanks, but not the 120% tank (Figure 3.7; 70%: χ^2 = 21.010, d.f. = 10 $P < 0.021$; 90%: χ^2 = 19.336, d.f. = 104, $P < 0.036$; 120%: χ^2 = 9.583, d.f. = 10, P = 9.583). In the 70% tank, a greater proportion of lower mode fish fed during the first two phases of the day and a smaller proportion fed during the last two phases of the day. In the 90% tank the effect was similar, but less pronounced, but in the 120% tank there was no difference in the proportion of fish of each size that fed at any point in the day. Small fish fed at similar times to large fish in the 70% tank, but at similar times to lower mode fish in the 120% tank (Figure 3.7).





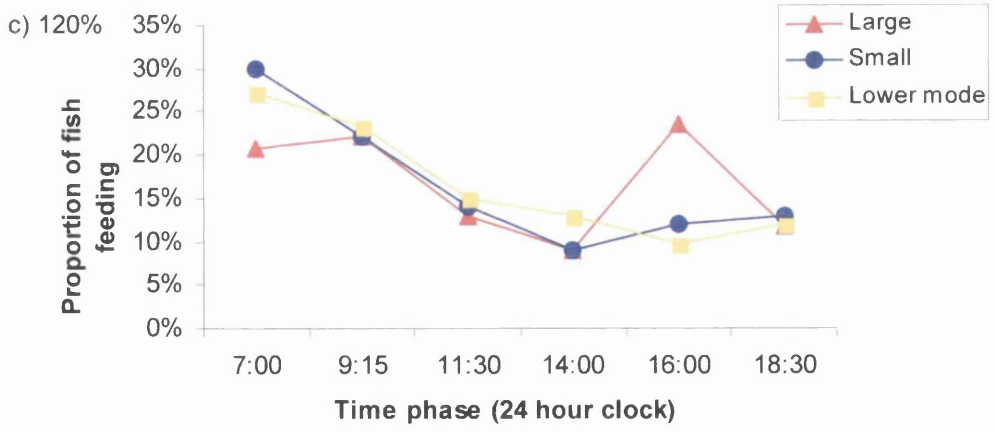


Figure 3.7 Proportion of lower mode, small and large fish feeding during different phases of the day in populations exposed to feeding regimes of a)70%, b)90%, and c)120% of commercial rations.

As there was a difference in the time of day that individuals of each category fed, the sequence of feeding was analysed with respect to time of day. This revealed no effect of time of day or category of fish on sequence of feeding in any tank (Table 3.2; Figure 3.8). However, a significant interaction between time of day and size-category on sequence of feeding was apparent in all tanks (Figure 3.8; Table 3.2). In the 120% tank, lower mode fish appear to feed first earlier in the day and large fish feed first later in the day. In the 90% tank the sequence of lower mode fish mirrors that of large fish, but lower mode fish feed first for most of the day. In the 70% tank, the trend observed in the 120% tank was reversed; large fish fed first earlier in the day and lower mode fish fed first later in the day. Small fish had a smaller degree of variation in the sequence that they fed in throughout the day than lower mode and large fish in all tanks.

Table 3.2 Summary of results from Scheirer-Ray-Hare (Dytham, 2000) tests on the effect of size category and time of day and their interaction on sequence of feeding.

Tank	Variable tested	χ^2	d.f.	*P*
70%	Size category	0.511	2,235	*0.225*
	Time of day	6.990	5, 235	*0.864*
	Size category*Time of day	1.004	10, ,235	*< 0.001*
90%	Size category	2.872	2,219	*0.762*
	Time of day	15.295	5,219	*0.991*
	Size category*Time of day	0.633	10,219	*< 0.001*
120%	Size category	0.873	2,246	*0.354*
	Time of day	11.349	5,246	*0.955*
	Size category*Time of day	0.753	10,246	*< 0.001*

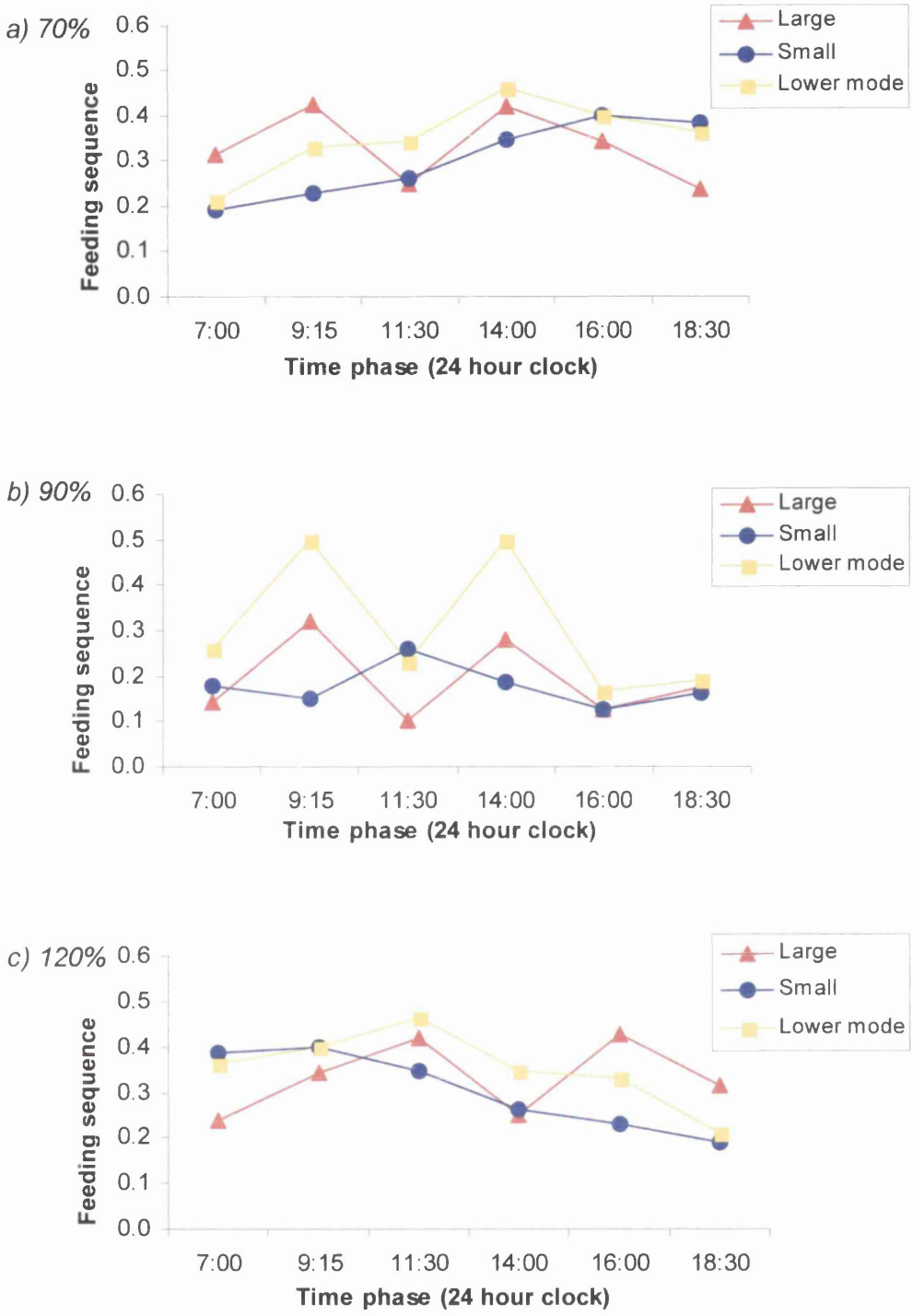


Figure 3.8 Median feeding sequence of lower mode, small and large fish during different phases of the day in populations exposed to a)70%, b)90% and c)120% of commercial rations. Interquartile ranges are omitted to aid clarity. N.B. Feeding sequence is a measurement of the point in the meal that an individual feeds (the time at which an individual fed divided by the total length of meal).

A significant relationship between the proportion of fish of each size attempting to feed and feeding regime was revealed (Figure 3.9; lower mode: $F_{1,385} = 14.956$, $r^2 = 0.04$, $P < 0.001$; small: $F_{1,385} = 26.398$, $r^2 = 0.06$, $P < 0.001$; large: $F_{1,386} = 26.939$, $r^2 = 0.07$, $P < 0.001$). For lower mode fish, a smaller proportion of individuals fed in the low feeding intensity condition and a larger proportion fed in the high feeding intensity. For large, and for small fish, the opposite was true; the proportion of fish attempting to feed tended to be higher in low feeding intensities and lower in high feeding intensities.

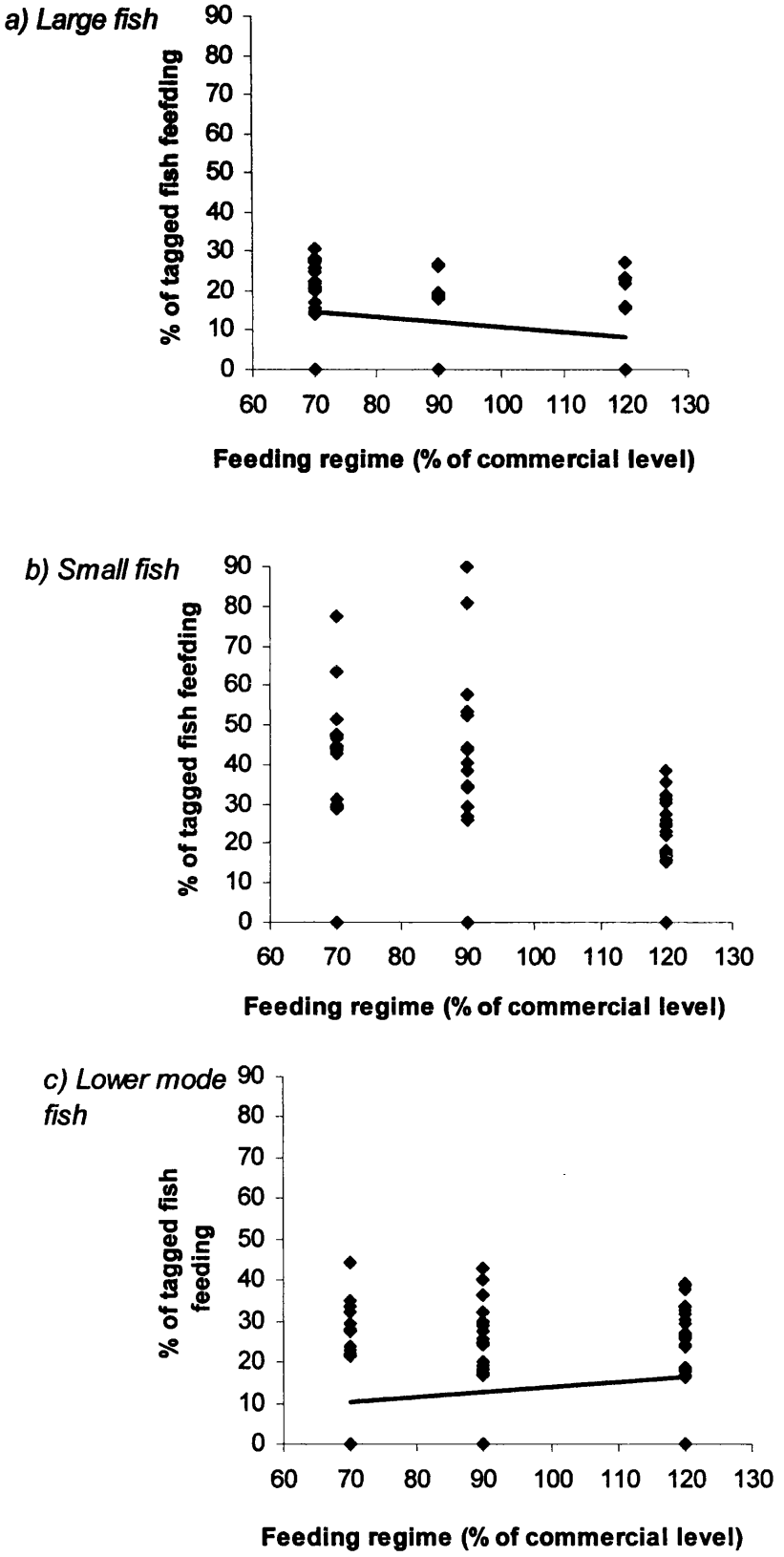


Figure 3.9 Effect of feeding regime on the proportion of a) large fish, b) small fish and c) lower mode fish feeding

3. 4 DISCUSSION

This study highlighted a number of differences in the behaviour of lower mode, small and large fish within a population. However, the method of tagging resulted in two major problems; a difference in the behaviour of tagged fish compared to non-tagged fish and a high degree of tag loss that was different for the three categories of fish studied.

3. 4. 1 Problems with tagging

In large groups of animals, identification of specific individuals within a population is necessary in order to study their behaviour. To allow continued identification of individuals it is often necessary to mark the subjects. Many different tags have been used to mark fish over the last 100 years; the majority of external tags are attached to the fish by implanting one end of the tag into the flesh of the individual (see McFarlane *et al.*, 1990 for review), but this can affect behaviour (Mourning *et al.*, 1994; Hughes, 1998). In the present study, tags were attached through the dorsal fin, to minimise healing and stress. However, this resulted in extensive and differential tag loss, indicating that this method of tagging was not suitable for large Atlantic salmon at high densities. During application of the tag, a small hole was made in the fin. Tag loss appeared to occur as a result of the hole in the fin becoming enlarged and the anchor bar slipping through it. The enlargement of the hole may have occurred during swimming, or during close contact with other fish during feeding.

As tag retention was higher in large fish, this suggests that different pressures were exerted on the fish of different sizes. Lower mode fish may have had thinner fins or have been exposed to more turbulent water flow due to differences in body size. Additionally, they may have been poorer competitors, and therefore may have frequently contested food with fish of superior strength and/or size. However, the finding that feeding intensity did not affect tag loss suggests that competition did not

affect tag loss. Very few incidences of aggression were observed during the video analysis, so it is unlikely that tag loss was caused by direct aggression.

A greater proportion of tagged fish than un-tagged fish were observed in the lower section of the tank. This may have been due to the method of data collection; as tagged fish may have been easier to spot than untagged fish in deeper water. When there were many fish on the screen, some were obscured by others, in contrast, it is likely that all tags would have been observed even if the whole fish was not visible. In addition, the observer was looking down on the bottom section, so tags in this section may have been easier to spot, as they would have been reflecting the light from above.

Alternatively, the tagged fish may have needed longer to recover from sampling than the other fish. Figure 3.4 indicates that this may have been the case, as the number of tagged fish increases over the first four days of observation. Although all subjects in each tank were sampled, the tagged fish would have been out of the water for longer during tag application. Jobling and Koskela (1996) reported that hierarchies in groups of Arctic charr were re-arranged after disturbance. Therefore, tagged fish may have had an initial disadvantage during hierarchy formation resulting in them being lower down the hierarchy.

3. 4. 2 Spatial distribution

In this study, large fish were more likely to occupy the top section of the tank, whereas lower mode fish were more likely to occupy the lower section. Food was supplied from the top of the tank, so the upper section was likely be the most profitable. Ryer and Olla (1996) noted that dominant coho salmon occupied the area closest to food introduction, and Kadri *et al.*, (1996a) demonstrated that the most successful fish in sea cages of Atlantic salmon (those that fed most) fed at the surface and contested many pellets, whereas less successful fish were found well below the surface and fed on pellets that dropped through the water column. Adams *et al.* (1998) also noted

different feeding strategies in juvenile Atlantic salmon, as some individuals swam quickly in and out of the feeding area in response to food. Therefore, the lower mode fish in the described study may have waited at the bottom for pellets that drifted down.

3. 4. 3 Feeding behaviour

Although food was dispersed throughout the tank by the water current, each delivery of food occurred in the same area each time. As predictable food sources are more likely to be monopolised (Jobling, 1995), larger individuals may have monopolised the upper section of the tank, forcing lower mode individuals to feed lower in the tank. The spatial distribution of both categories of fish from the main mode (small and large) of the weight distribution, was similar in all feeding regimes. In large groups, dominance hierarchies are likely to be less stable (Fenderson & Carpenter, 1971) so there may have been less of a behavioural distinction between small and large individuals within the groups.

There was a difference in the time of day that lower mode and large fish fed during the study. This difference was most apparent in the lowest feeding regimes. Indeed, there was no significant difference between the time of feeding attempt in the three groups in the highest regime. In the 70% feeding regime lower mode fish fed to a greater extent earlier in the day and to a lesser extent later in the day. Large fish displayed the opposite pattern. Kadri *et al.* (1997) also found that post smolt Atlantic salmon of different sizes fed at different times of the day (in the laboratory); small fish fed at first and last light under a light regime where the lights went on at 08.00 and off at 20.00 in May. Failure to observe lower mode fish feeding at the end of the day in the present study may have been due to the lack of feeding opportunities at this time. In the present study the fish were exposed to artificial light from 07.00 until 19.00, but the natural photoperiod was longer than this (in July / August). As the occurrence of circannual growth rhythms have been noted under the described conditions (chapters

2, 5 and 6), the lower mode fish may not have had the opportunity to feed at the end of the day.

Large and small fish were observed to feed in a similar pattern to that observed in sea cages (Kadri *et al.*, 1991; Blyth *et al.*, 1993); feeding peaked in the morning, decreased in early afternoon and increased again in late afternoon. As this was almost the opposite to lower mode fish, lower mode fish may therefore have been forced to feed at different times from other fish. Studies on juvenile salmonids have shown that dominants tend to feed at different times of the day (Harwood *et al.*, in press) and more preferred times of the day (Alanärä *et al.*, 2001).

Differences were also found in the sequence that fish of different size categories fed (within a feed delivery), with the differences being affected by time of day. Lower mode fish fed earlier in the feed delivery during the first two phases of the day and later in the feed delivery in the last two phases of the day. Lower mode fish may have a greater motivation to feed earlier in the day when there are less large fish feeding.

The observed differences in feeding sequence and in time of feeding during the day were more pronounced in low feeding regimes. This suggests that competition was higher in the lower feeding regimes. As there was no difference in the extent of tag loss in the different regimes, and as the groups had been established for some time (since the start of part 1), it is likely that feeding hierarchies or behavioural strategies were more pronounced when feed levels were lower but the actual levels of direct aggressive competition were low.

This study demonstrated a clear difference in the behaviour of lower mode and large fish from the main mode. These differences were increased in lower feeding regimes and less apparent in high feeding regimes. Small fish from the main mode were similar to large fish with respect to the position that they held in the tank, but they also showed

some differences with respect to feeding behaviour. In particular, the small fish showed less variation in the sequence in which they fed. In the current study, sequence of feeding is likely to be a good indicator of feeding motivation and willingness to compete for food. Fish that feed first would have an increased motivation and willingness to compete for food. As small fish were generally later in the sequence than at least one category of fish they may be opting for a less competitive strategy of feeding.

The proportion of fish of each category that fed during a feed delivery were examined with respect to feeding regime. A greater proportion of large fish fed in lower feeding regimes than higher feeding regimes, whereas a smaller proportion of lower mode fish fed in lower feeding regimes. As larger fish are better competitors (all fish were originally the same size), they may outcompete smaller fish in lower feeding regimes. Competition is more relaxed in higher feeding regimes so this may enable the lower mode fish to feed. Magnuson (1962) also found that low ranked Medaka had more access to food when it was more abundant. Furthermore, studies on brown trout (Alanärä *et al.*, 2001) and Eurasian badgers (Revilla & Palomares, 2001) demonstrated that when demand for food increased, dominants increased their access to food, whereas access of subordinate individuals was decreased.

Behaviourally distinct categories of dominant and subordinate salmonids are well documented (Jenkins, 1969; Metcalfe, 1986; O'Connor, 2000). Recently, Höjesjö *et al.* (in press) identified three categories of brown trout by aggressive interactions displayed in dyadic encounters; dominant, subordinate and non-aggressive individuals. The non-aggressive individuals showed less variation in stream position than subordinates and dominants and were believed to be generalists. Similarly, in a study where Arctic charr were presented with a choice of two feeding tanks, Brännäs and Eriksson (1999) reported three categories of individuals; 'floating, switching and non-switching'. Floating individuals were found mostly in the non-feeding tank, switchers visited both

tanks repeatedly and non-switching individuals always visited the same feeding tank. Floaters and switchers had the lowest growth rates and switchers were suggested to have a lower competitive ability. The small fish in the current study may behave in a similar fashion to the non-aggressive or floating individuals found by Höjesjö *et al.* (in press) and Brännäs and Eriksson (1999) as they appear to compete for food less extensively and may feed opportunistically; depending on the activities of the other fish in the tank.

3. 4. 4 Conclusions and implications

This study presented evidence that lower mode fish appear to be excluded from the most profitable feeding sites (in the upper section of the tank), especially when groups are exposed to low rations and are forced to feed at times when larger, more dominant fish are not actually feeding.

The results of the study confirm the occurrence of behaviourally distinct groups of fish in large groups. In addition, even at high rations there is a subclass of individuals that seem to be behaviourally excluded from feeding and are forced to feed opportunistically or not at all. The following chapter investigates the occurrence of social hierarchies within these groups of Atlantic salmon through the application of a biochemical tool.

CHAPTER 4. A BIOCHEMICAL TOOL FOR RECONSTRUCTING SOCIAL EXPERIENCE; APPLICATION TO FARMED ATLANTIC SALMON.

4. 1 INTRODUCTION

Interest in the welfare of captive animals, reared in large numbers for research, food and clothing, has increased dramatically in recent years. One of the principal sources of stress experienced by animals in large groups is their conspecifics (Webster, 1995). For example, free-range chickens, released from the stresses of battery farming, are denied access to particular areas by dominant individuals (Grigor *et al.*, 1995). Dominance hierarchies result from imbalances in competitive ability and resource acquisition and can result in increased stress levels in both subordinate and dominant animals (Winberg & Lepage, 1998; Creel *et al.*, 1999). Social stress has wide ranging effects, including decreased immunocompetence in vertebrates (Heise & Van Acker, 2000), including humans (Vuitton *et al.*, 1999), and impaired digestion in fish (Olsen & Ringø, 1999). Understanding social stress within groups is important in improving both production and welfare in man-made groups of animals and in the conservation of natural groups.

The quantification of behaviour in large groups is conceptually simple, but technically very challenging. Previous methods have utilised video (see chapter 2), hydro acoustics (reviewed by McLennan & Simmonds, 1992) and acoustic telemetry (Juell & Westerberg, 1993; Bégout-Anras, 1995). However, difficulties still prevail in the identification and tracking of known individuals at high densities. Indexes of

performance and resource allocation have been formed from various parameters e.g. individual meal size, growth and size heterogeneity (Carter *et al.*, 1992; McCarthy *et* al., 1992a; Damsgård *et al.*, 1997), but these measures can only make indirect inferences about the behaviour of individuals.

Knowledge of the physiological basis of many behaviours is increasing, promoting the combined use of behaviour and physiology in welfare research (Rushen & de Passille, 1998). One possible solution, in the present context, is to utilise biochemical indicators to identify socially stressed individuals *post hoc*. Stress responses in the endocrine system are highly conserved throughout vertebrates, with increased levels of circulating blood glucocorticoids reported in subordinate rainbow trout and baboons (Sapolsky, 1990; Noakes & Grant, 1992). Recently, cortisol has been shown to be highly variable and context dependent (Creel *et al.*, 1999; Summers, 2001) and therefore may not give an accurate picture of long-term chronic social stress.

Brain neurotransmitters are another important indicator of stress, as they are believed to be involved in the control and integration of behavioural and physiological stress responses in both fish and mammals (Höglund *et al.*, 2000). The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) modulates aggression in many species. Serotonin inhibits aggressive vocalisations in cats (Golebiewski & Romanick, 1985), whilst other studies have found reduced levels in human suicide victims (reviewed by Mann *et al.*, 1989). The ratio of brain tissue concentrations of 5-hydroxyindoleacetic acid (5-HIAA, the main serotonin metabolite) to 5-HT (5-HIAA/5-HT ratio) has been used as a remarkably sensitive index of chronic social stress in salmonid fish in pairs or small groups. Individuals defeated in agonistic encounters display a highly characteristic increase in serotonergic activity, indexed by the ratio of 5-HIAA/5-HT in various brain regions including the brain stem (Winberg *et al.*, 1992a). This increase has been used to re-create the social history of individuals in groups of up to four salmonids under laboratory conditions (Winberg & Nilsson, 1993). Furthermore, the 5-

HIAA/5-HT ratio was found to correlate with the number of aggressive attacks received by subordinates (Winberg & Lepage, 1998).

Salmonid fish farming is a relatively new industry and therefore provides the opportunity for modern science to investigate the process of domestication. New techniques, hitherto unknown, allow a much greater understanding of the changes in physiology and behaviour taking place, and the mechanisms behind these. In farmed salmonids, large differences in individual growth rates suggest the occurrence of competition and unequal resource partitioning (Jobling & Koskela, 1996; Volpato & Fernandes, 1994). This is counter-intuitive as one might expect dominance hierarchies to break down when group sizes increase and resources become harder to defend (Noakes & Grant, 1992). However, recent studies (chapters 2 & 3), indicate that marked and persistent differences in growth rates may be the result (in part at least) of behavioural interactions. Therefore, information on the potentially stressful effects of aggressive resource acquisition is needed in order to assess welfare and improve growth in both aquaculture and agriculture. In the experiment described here, farmed fish were used as a case study to investigate the presence and form of dominance hierarchies in large groups, using brain serotonergic activity as an index of previous social experience. In addition, as one might expect dominance hierarchies to be intensified when competition for resources is increased, the effect of competitive intensity on these hierarchies was also investigated.

4. 2 METHODS

4. 2. 1 Overall strategy

The subjects for this study were commercially farmed adult Atlantic salmon, initially tightly size-graded, allowing body weight to be used as a proxy for performance. The fish were held, for at least 11 weeks, in conditions of high or low competitive intensity,

created by varying the level of feed availability. Serotonergic activity, as indexed by the 5-HIAA/5-HT ratio, was measured in the brain stem of 55 Atlantic salmon, 20 from highly competitive regimes and 35 from regimes with a low competitive intensity.

4. 2. 2 Subjects and experimental conditions.

The study was carried out at EWOS Innovation Forskningstassjon, Lønningdal, Norway. 1200 fish from the same stock, with a weight range of 1.34kg ± 3%, were transferred from 15m x 15m x 15m deep sea-cages into 10 circular seawater tanks in November 1999. The tanks had a water depth of 1.5m and a diameter of 3m, with a median temperature of 8.4 °C (range: 7.6 °C - 11.5°C). Each tank contained 120 fish, giving a relatively high density of 15.2kg.m^{-3}; densities in commercial farming of Atlantic salmon range from 12-15kg.m^{-3}. Seawater entered the tank at 210 l.min^{-1} through a vertical pipe at the side, and exited from the centre, giving a circular flow. The oxygen level in the outlet ranged from 75 to 80% saturation and the light regime was 12h light: 12h dark for the duration of the experiment. Following 1 month of acclimation, during which time fish were fed on commercial feeding regimes, 60 fish from each tank were fitted with VI tags (Northwest Marine Technology Ltd. Shaw Island WA; Niva, 1995). The experimental feeding regimes began in January 2000; following 2 weeks of acclimation to ensure the tagged fish had recovered. During the study, the timing of sampling is referred to in weeks from tagging, not from the start of the experimental feeding regimes. The feeding regimes were derived from a value used in commercial conditions of 0.5% body weight per day (bwpd); 3 tanks were fed at this level. 2 tanks were fed on a maintenance ration of 0.15%bwpd, (30% of the commercial ration) and 3 tanks were fed on levels between maintenance and commercial rations: 0.25%bwpd, 0.35%bwpd and 0.45%bwpd, giving 50%, 70% and 90% of the commercial ration respectively. The remaining two tanks were fed to excess on 0.6%bwpd, 120% of the commercial ration. Commercial feed was used for the duration of the trial (EWOS Dynamic Red, size L) and was released from pipes spanning the width of the tank, every 7.5 minutes, with lower regimes having shorter meals. For the purpose of

analysis feed levels of 30% and 50% were referred to as high competitive intensity and feed levels of 90%, 100% and 120% were referred to as low competitive intensity. Feed intensities in low competitive conditions were representative of those found in aquaculture, while all rearing densities were in the top range of those used commercially.

4. 2. 3 Sampling and biochemical analysis.

60 fish were sampled for the present study at two sampling points, 36 fish at 13 weeks, and 24 fish at 29 weeks. At 13 weeks, fish were taken from all tanks except that fed on the 70% feeding regime. At 29 weeks, fish were taken from two tanks fed on 30% feeding regimes, two tanks fed on 100% feeding regimes and two tanks fed on 120% feeding regimes. All sampling was carried out at midday. Four fish were netted from each tank, anaesthetised (500mg/l ethyl *m*-aminobenzoate methane-sulphonate), weighed, measured and blood sampled before decapitation. Attempts were made to choose large and small fish, but priority was given to the speed of catching rather than selection of individuals. A heparinised syringe was used to collect blood from the caudal vasculature for cortisol analysis, it was then spun at 3000rpm, and the plasma removed and frozen on dry ice. All samples were removed and frozen within 3 minutes of the fish dying and 15 minutes after removal of the fish from the tank. The gender of each fish was identified following tissue collection. Brain stem samples were wrapped in aluminium foil, frozen in liquid nitrogen and kept at -70°C until shipment from Bergen to Uppsala on dry ice for analysis. Brain levels of 5-HT and 5-HIAA were analysed using HPLC with electrochemical detection (Øverli *et al.*, 1999b). The ratio of 5-HIAA to 5-HT concentration was calculated and used as an index of brain serotonergic activity. This is a more direct index of serotonergic activity than brain levels of 5-HIAA *per se*, since variance related to tissue sampling, and differences related to total levels of 5-HT and 5-HIAA, are reduced (Shannon *et al.*, 1986). Plasma cortisol analysis was carried out using a radioimmunoassay described by Olsen *et al.* (1992). Three

samples were lost and two were identified as erroneous and removed from the analysis.

4. 2. 4 Growth rates

Growth was calculated for tagged individuals using Thermal Growth Coefficient (TGC) according to the following formula (Cho, 1992):

$$TGC = ((W_{t1}^{1/3} - W_{t0}^{1/3}) / T \times t)1000$$

Where W_{t0} represents weight in grams at time 0, W_{t1} weight in grams at time 1, T the mean temperature in °C, and *t* the number of growth days.

4. 2. 5 Preliminary analysis.

There was no relationship between fish size and growth rate in the populations fed at 100% that provided the fish for this study at either sampling point (13 weeks: $F_{1,61}$ = 2.820, R^2 = 0.10, *P* = 0.245; 29 weeks: $F_{1,129}$ = 1.362, R^2 = 0.44, *P* = 0.098). The effect of sampling date (after 13 or 25 weeks) on 5-HIAA/5-HT ratio was marginally non-significant (independent samples t-test: t_{53} = 1.89, *P* = 0.06) but there was no effect of sampling date on the relationship between 5-HIAA/5-HT ratio and weight (ANCOVA: comparison of regression slopes $F_{1,51}$= 0.148, *P* = 0.702). There was no effect of sequence in which the four fish were removed from each tank (one-way ANOVA: $F_{3,54}$ = 0.38, *P* = 0.77), nor was there an effect of gender (independent samples t-test: t_{45} = 0.16, *P* = 0.87) on the brain stem serotonin turnover, so males and females were pooled for analysis. The 5-HIAA/5-HT ratios found in this study have a similar range as those previously found in smaller fish (Winberg *et al.* 1992a; Winberg & Lepage 1998; Øverli *et al.* 1999b), indicating that fish size does not directly affect 5-HIAA/5-HT ratio.

4. 3 RESULTS

Firstly, the relationship between plasma cortisol concentration and brain stem serotonin turnover was examined; this revealed no relationship ($F_{1,52} = 0.08$, $R^2 = 0.00$, $P = 0.78$). In addition there was no relation between cortisol concentration and weight ($R^2 = 0.02$, $F_{1,57} = 1.23$, $P = 0.27$), TGC ($F_{1,27} = 0.55$, $R^2 = 0.01$, $P = 0.46$) or regime level ($t_{57} = 1.31$, $P = 0.20$).

In contrast, there was a strong relationship between brain stem 5-HIAA/5-HT ratios and performance, with small and slow growing fish having high 5-HIAA/5-HT ratios, whilst large and fast growing fish had low 5-HIAA/5-HT ratios. This relationship was found under both high and low competitive conditions, indicating that dominance relationships were maintained even when resources were plentiful. There was, however, a difference in the structure of the relationship between brain biochemistry and size in high and low competitive intensities. The relationship between weight and 5-HIAA/5-HT ratio for fish from low competitive conditions was best explained by an exponential regression (Figure 4.1; $R^2 = 0.48$, $F_{2,32} = 14.83$, $P < 0.001$); medium and large sized fish had similar 5-HIAA/5-HT ratios and small fish had higher 5-HIAA/5-HT ratios. Subjects from high competitive conditions displayed a linear relationship between weight and 5-HIAA/5-HT ratio ($F_{1,19} = 5.35$, $R^2 = 0.23$, $P = 0.03$), indicating that medium sized fish had a higher 5-HIAA/5-HT ratio than larger fish. In addition, fish from high competitive conditions had a significantly higher 5-HIAA/5-HT ratio than fish from low competitive conditions (independent samples t-test: $t_{53} = 0.412$, $P < 0.001$, mean and standard error (SE); high = 0.305 ± 0.012; low = 0.248 ± 0.009).

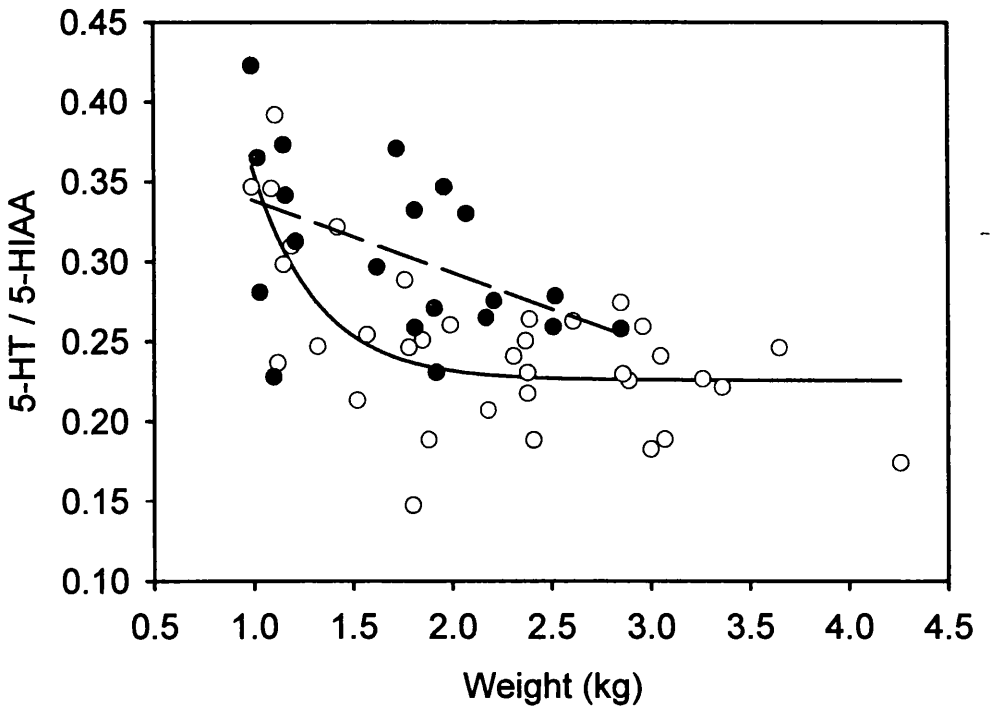


Figure 4.1 The relationship between weight and 5-HIAA/5-HT ratio for fish from conditions of high competition (filled circles; linear dashed line) and low competition (hollow circles; exponential solid line; $y = a + b(e^{-x/c})$, where $a = 0.23$, $b = 2.85$, $c = 0.32$).

There was no relationship between growth rate and 5-HIAA/5-HT ratio in subjects from high competitive intensities (Figure 4.2; $F_{1,7} = 1.431$, $P = 0.270$). However, in subjects from low competitive intensities there was a significant negative relationship between growth and 5-HIAA/5-HT ratio (Figure 4.2; $F_{1,16} = 12.490$, $P = 0.003$).

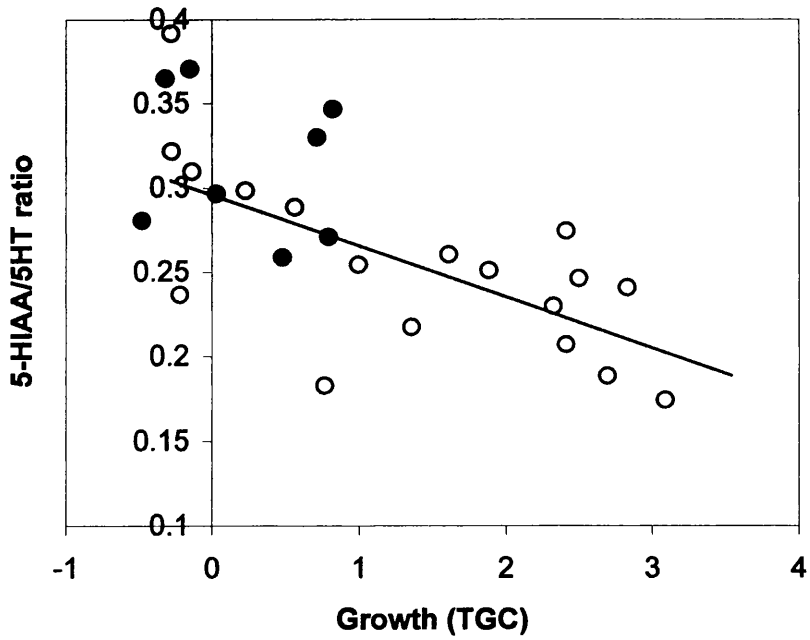


Figure 4.2 The relationship between growth (TGC) and 5-HIAA/5-HT ratio for fish from conditions of high (filled circles) and low (hollow circles, solid line) competition.

4. 4 DISCUSSION

4. 4. 1 Cortisol analysis

In the described study, there was no relationship between cortisol levels and performance, irrespective of feeding regime. This differs from a number of other studies that have found increased glucocorticoid levels in subordinate individuals immediately following a period of agonistic behaviour (e.g. Winberg & Lepage, 1998). However, it does agree with results from studies by Øverli *et al.* (1999b), who found that cortisol levels in rainbow trout returned to base levels in long established hierarchies, and by Schreck (1981), who found that cortisol levels eventually return to pre-stress levels during chronic stress. The groups used in the present study were formed at least 8 weeks prior to sampling, possibly resulting in relatively stable hierarchies at the time of sampling. Furthermore, as the process of becoming

4. 4. 2 Relationship between weight and 5-HIAA/5-HT ratio

Conversely, there was a clear relationship between 5-HIAA/5-HT ratio and weight in both high and low competitive conditions, with a higher 5-HIAA/5-HT ratio in small, slow growing fish and a lower 5-HIAA/5-HT ratio in large, fast growing fish. Since Winberg and colleagues have shown that rates of serotonin turnover are specifically related to the experience of low social status (Winberg *et al.*, 1991; Winberg *et al.*, 1992a; Winberg & Lepage, 1998; Øverli *et al.*, 1999b), these results strongly suggest that Atlantic salmon held at high densities display social polarisation and that social status determines access to food.

The structure of this hierarchy appears to change with the intensity of competition. The exponential regression explaining the relationship between 5-HT and weight in low competitive conditions showed a similar level of social stress in medium and large sized fish and a higher 5-HIAA/5-HT ratio in small fish. These results suggest the existence of dominance hierarchies with a category of profoundly subordinate fish experiencing behavioural and growth suppression, even in large and dense groups with a surplus of food. Alanärä *et al.* (1998) also found an equivalent pattern; lower serotonin turnover rates in intermediate and dominant Arctic charr, and higher rates in subordinates, in groups of 8 fish fed by a demand feeding system. The subordinate fish probably experience attacks or intimidation, resulting in either restricted access to food, or stress induced appetite inhibition. In contrast, Winberg *et al.* (1991) and Winberg and Nilsson (1996) found significantly lower rates of serotonin turnover in dominant rainbow trout compared to those of intermediate status.

The literature reports both linear hierarchies (Fausch, 1984; Noakes & Grant, 1992; Winberg *et al.*, 1992a) and non-linear hierarchies (Alanärä & Brännäs, 1996; Kristiansen, 1999) in salmonid fish depending on environmental conditions, including group size. It is possible that, at the high densities of the present study, it is difficult for one fish alone to be dominant; several dominant individuals may exist with a very small

distinction between dominant and intermediate fish. However, the individuals at the lower end of the hierarchy would still be threatened continuously, hence the fact that their brain biochemistry shows clear signs of social subordination.

Under high competitive conditions, a linear relationship was found between weight and 5-HIAA/5-HT ratio, revealing the presence of a linear dominance hierarchy, directly related to weight. It would seem that under conditions of increased competition, a hierarchy is established between medium and large sized fish. In conditions of increased competition, there was an increase in the 5-HIAA/5-HT ratio in fish of all sizes and a greater number of fish with 5-HIAA/5-HT ratios characteristic of subordinates. As food deprivation *per se* does not increase brain serotonergic activity (Winberg *et al.*, 1992b), the dominance hierarchy appeared to intensify and become more polarised when competition was increased. Previous studies, on small groups of salmonids, have documented that dominance hierarchies become more pronounced when competition is increased (e.g. Noakes & Grant, 1992; McCarthy *et al.*, 1992a; Winberg *et al.*, 1993; Carter *et al.*, 1996).

4. 4. 3 Relationship between growth and 5-HIAA/5-HT ratio

In contrast to the negative relationship found between weight and the 5-HIAA/5-HT ratio, there was no relationship between growth in the period prior to sampling and the 5-HIAA/5-HT ratio in high competitive conditions. This may have been influenced by the small sample size (8 individuals). Alternatively, there may have been smaller differences between the social stress levels of fish in high competitive conditions resulting in an increase sampling in the 5-HIAA/5-HT ratio, but no clear pattern between growth and 5-HIAA/5-HT ratio.

Individuals from low competitive intensities displayed a difference in the relationship between growth and 5-HIAA/5-HT ratio and the relationship between weight and 5-HIAA/5-HT ratio. This could also have been caused by a decrease in the sample size,

as complex relationships (such as exponential regressions) are more difficult to ascertain in lower sample sizes. Alternatively, this result may indicate that social hierarchies in large groups may be linear with respect to growth, but non-linear in terms of weight; there is a sub-group of fish with a markedly higher 5-HIAA/5-HT ratio than the remaining fish in terms of weight. As individuals in the described study were individually marked, growth is the most accurate measure of performance, however growth data is only available on a much smaller subset of individuals than weight data.

The densities used in this study were high and the high competitive conditions are more intense than those found in Atlantic salmon farming. Nonetheless, the results emphasise the importance of feed management in aquaculture, showing that a decrease in feeding level is detrimental to all individuals irrespective of social status, and that optimum feeding regimes should not restrict feed. Previous studies have noted that farmed fish are not always fed to their natural appetite rhythms (Jobling *et al.,* 1995; Juell, 1995), which results in underfeeding and consequently increased social stress. Assuming the pattern of social stress detected in the low competition treatments are representative of commercial conditions, there are a small percentage of commercially farmed salmonids that suffer social stress related to competition. In the tanks on low competitive regimes that provided the fish for this study, 12% of fish did not grow. Hence, mechanisms that decrease social stress and therefore the number of subordinate individuals will improve both the welfare of the fish and the commercial returns to the farmer.

In terms of fundamental behavioural biology, these results demonstrate that structured social hierarchies exist and determine access to resources even when animals are held at very high densities; the detailed manifestations of these hierarchies may be different but the behavioural processes appear to be the same. Dominance-subordinance relationships allow individual salmon that are severely out-competed to keep out of trouble while feeding opportunistically and so represent an adaptive strategy in

but the behavioural processes appear to be the same. Dominance-subordinance relationships allow individual salmon that are severely out-competed to keep out of trouble while feeding opportunistically and so represent an adaptive strategy in subordinate fish, at low densities under natural conditions (Metcalfe, 1996), and possibly at high densities as well. Finally, this study has shown that brain bioamines can be used as a tool to reconstruct recent social experience, and therefore assess welfare, in conditions where direct behavioural observation is not possible. As the neuroendocrine system is highly conserved among vertebrates, this technique could be applied in vertebrate groups other than fish.

4. 4. 4 Conclusions and implications

This study demonstrated that poor growing fish had a 5-HIAA/5-HT ratio similar to that of subordinate individuals. While it remains possible that lower mode fish are voluntarily abstaining from feeding, or that they are less efficient at processing feed, this study illustrates that behavioural interference can suppress feeding. This raises the issue of welfare in farmed fish and suggests that ways of minimising this effect should be investigated.

Two Atlantic salmon, exposed to the same ration for 29 weeks

CHAPTER 5. SEPARATION OF NON-GROWING FROM GROWING FISH; COMPENSATION FOR LOST GROWTH OPPORTUNITIES

5. 1 INTRODUCTION

Size variation is a problem in the aquaculture industry, which aims for consistently high quality products. Fish of different sizes have different feed requirements and small, poor-growing fish are unmarketable, as they have little meat and are thin and unattractive. Differences in size at harvesting in initially-graded fish result from differential growth during production.

Size heterogeneity has previously been reduced by routine size-grading at various stages throughout production, but this practice is declining in popularity. Originally, size grading was thought to disrupt hierarchies, allowing smaller fish to grow and improving feeding over time (see Jobling & Reinsnes, 1987; Wallace & Kolbeinshavn, 1988), but it temporarily stresses fish, resulting in the loss of feeding days. There is also conflicting evidence on the effectiveness of size-grading, as many studies have failed to show benefits in terms of biomass gain (Jobling & Reinsnes, 1987; Wallace & Kolbeinshavn, 1988; Baardvik & Jobling, 1990; Jobling & Baardvik, 1994; Stefánsson *et al.*, 2000). However, there may be benefits for particular fish within the size distribution, as studies on sea bream (Umino *et al.*, 1994), sturgeon (Georgiadis *et al.*, 2000a; Georgiadis *et al.*, 2000b), Arctic charr (Jobling & Reinsnes, 1987) and prawns (Karplus *et al.*, 1992) have shown that small individuals show improved growth in the absence of larger individuals.

During grading, it is common practice to remove and kill the smallest fish, as they are thought to have an inherently low growth potential and increased susceptibility to disease. Increased knowledge of the characteristics and possible growth of these smaller fish, or 'runts' upon separation from larger fish would provide information on the mechanisms responsible for poor growth in aquaculture and allow recommendations to be made on the validity of size-grading.

Growth variation appears to have a genetic basis, as it is found in individuals grown in isolation (e.g. juvenile sunfish, Wang *et al.*, 1998; rainbow trout, Mäkinen & Ruohonen, 1992), but the extent of variation is increased in group-reared animals, implying that social interactions also effect growth rates (Koebele, 1985; Volpato *et al.*, 1989; Miglavs & Jobling, 1989; Jobling, 1995; Wang *et al.*, 2000). Extensive investigation of cultured fish has identified many factors that influence the extent of differential growth, including feed levels and social interactions. Previous chapters of this thesis suggest that there is a subset of fish that may grow poorly because of social subordination. Therefore, it is of interest to investigate the growth of these individuals upon separation from larger fish.

One might expect fish that obtain the greatest amount of food show the highest growth rates. When fish are held in groups, individuals gain more food through competition, which may be either direct or indirect. In conditions of direct competition, the best competitors obtain the greatest proportion of food at each opportunity (Baardvik & Jobling, 1990; Kristiansen, 1999). In contrast indirect competition involves intimidation, in the form of visual, physical, or chemical cues, resulting in behavioural suppression in subordinates and increased unchallenged access to food for dominant individuals (Abbott *et al.*, 1985; Jobling, 1985; Koebele, 1985; Matty, 1986).

Irrespective of feed intake, the growth of subordinate individuals can also be suppressed by other mechanisms. Social intimidation itself can suppress growth of subordinates, as it results in high levels of social stress for subordinate fish (Noakes & Leatherland, 1977; Ejike & Shreck, 1980; Jobling & Reinsnes, 1987; Volpato *et al.*, 1989). Such stress can have wide-ranging effects, including increased disease susceptibility (Pickering, 1992), reduced healing ability (Nicieza & Metcalfe, 1999) and decreased appetite and digestive efficiency (Jobling & Wandsvik, 1983; Abbott & Dill, 1989). Furthermore, subordinate individuals may have relatively higher activity levels (Grant & Noakes, 1988; Fausch, 1984), reducing the amount of energy available for growth. Size variation is also heavily affected by feeding regimes; lower and more defensible feeding regimes result in increased competition for resources, which in turn lead to increased size variation (McCarthy *et al.,* 1992b; Ryer & Olla, 1996; Grant, 1993; Jobling, 1995; Jobling & Koskela, 1996).

In a wide range of species, suppression of growth rates by low feed rations results in compensatory growth responses when access to food improves (e.g. sheep and cattle, Ryan *et al.*, 1993; broiler chicken, Zubair & Leeson, 1996; grasshopper mice, Sikes, 1998; Arctic charr, Jobling *et al.*, 1993). However, in salmonids, periods of low temperatures can also induce compensatory growth (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997; MacLean & Metcalfe, 2001). Therefore, it is possible that individuals that have undergone decreased growth levels because of competition for food or growth suppression could also undergo compensatory growth.

The mechanisms behind the initiation of compensatory growth are not completely clear, but it appears to be controlled by the ratio of fat stores to structural tissue (Jobling & Miglavs, 1993; Broekhuizen *et al.*, 1994; Johansen *et al.*, 2001). In addition, as wild salmonids undergo seasonal changes in growth and condition (e.g. Gardiner & Geddes, 1980) it is likely that compensatory growth may be a result of a comparison of absolute body size with a target size for the time of the year (MacLean & Metcalfe,

2001). Daylength is an important cue in circannual rhythms in salmonids (reviewed by Boeuf & Le Bail, 1999). The current study was carried out at the time of the summer solstice and therefore provided the opportunity to relate the growth of fish of different performance (growing and non-growing) to season.

During a previous experiment, persistent and marked size differences were observed in groups of Atlantic salmon held at commercial densities (Chapter 2). This provided the opportunity to examine the development of fish that did not grow and to characterise their performance. Additionally, at the end of the experiment, the opportunity arose to examine the effects of social interaction and season on these non-growing individuals.

5. 1. 1 Aims

The aims of the described study were to identify non-growing Atlantic salmon from populations grown in semi-commercial conditions and to characterise their development. In addition, the effects of season and of larger fish on the growth of these non-growing individuals were determined. To do this, the growth of two groups of previously non-growing fish were compared whilst grown in groups with mixed sizes of fish and in a group of similar sized only-non-growing fish, as natural daylength started to decrease (following the summer solstice). The populations of previously non-growing fish that were grown in groups of mixed sizes were not complete controls for those that were grown in groups of similar-sized non-growing fish, but they did provide a useful comparison that allowed the following questions to be addressed:

- To what extent do non-growing fish differ from growing fish in terms of weight, length and condition?
- How does feeding intensity affect the number and condition of non-growing fish?
- Do fish that have not grown in the presence of growing fish start to grow when they are placed in a population consisting only of other non-growing individuals?
- Do injuries affect the growth of previously non-growing fish?

- If the previously non-growing individuals do grow, is this a result of removal from growing fish or influenced by seasonal cues?

The experiment consisted of two parts:

Part 1. A differential growth period of 29 weeks (see chapter 2), during which populations of fish were grown on a range of feeding regimes, each generating fish that did not grow.

Part 2. An investigation period of 17 weeks, split into two phases, that began at the summer solstice. The performance of non-growing fish was monitored, both in the groups of mixed sizes and in groups of similar-sized non-growing fish.

5. 2 METHODS

5. 2. 1 Experimental housing

Throughout the study, the fish were housed in circular, 2m deep x 3m diameter experimental tanks. Seawater was supplied from a depth of 30m, providing an ambient water temperature. In part 1 the median temperature was 8.4°C (range: 6.4 - 10.5°C (Figure 5.1). In part 2 the median temperature was 8.1°C (range: 7.5 - 11.5°C) until week 8 (phase I). For the remaining 9 weeks (phase II), the median temperature was 8.4°C (Figure 5.1; range: 6.4 - 10.5°C). Water entered the tank at 210 $l.min^{-1}$, through a vertical pipe at the side and exited in the centre, giving a circular flow. The oxygen level in the outlet water ranged from 75 to 80% saturation and the subjects were exposed to a photoperiod of 12h light and 12h dark for the duration of the experiment. Additionally, a small amount of natural light entered the tank through a 30cm diameter window on the tank wall, 1m from the base.

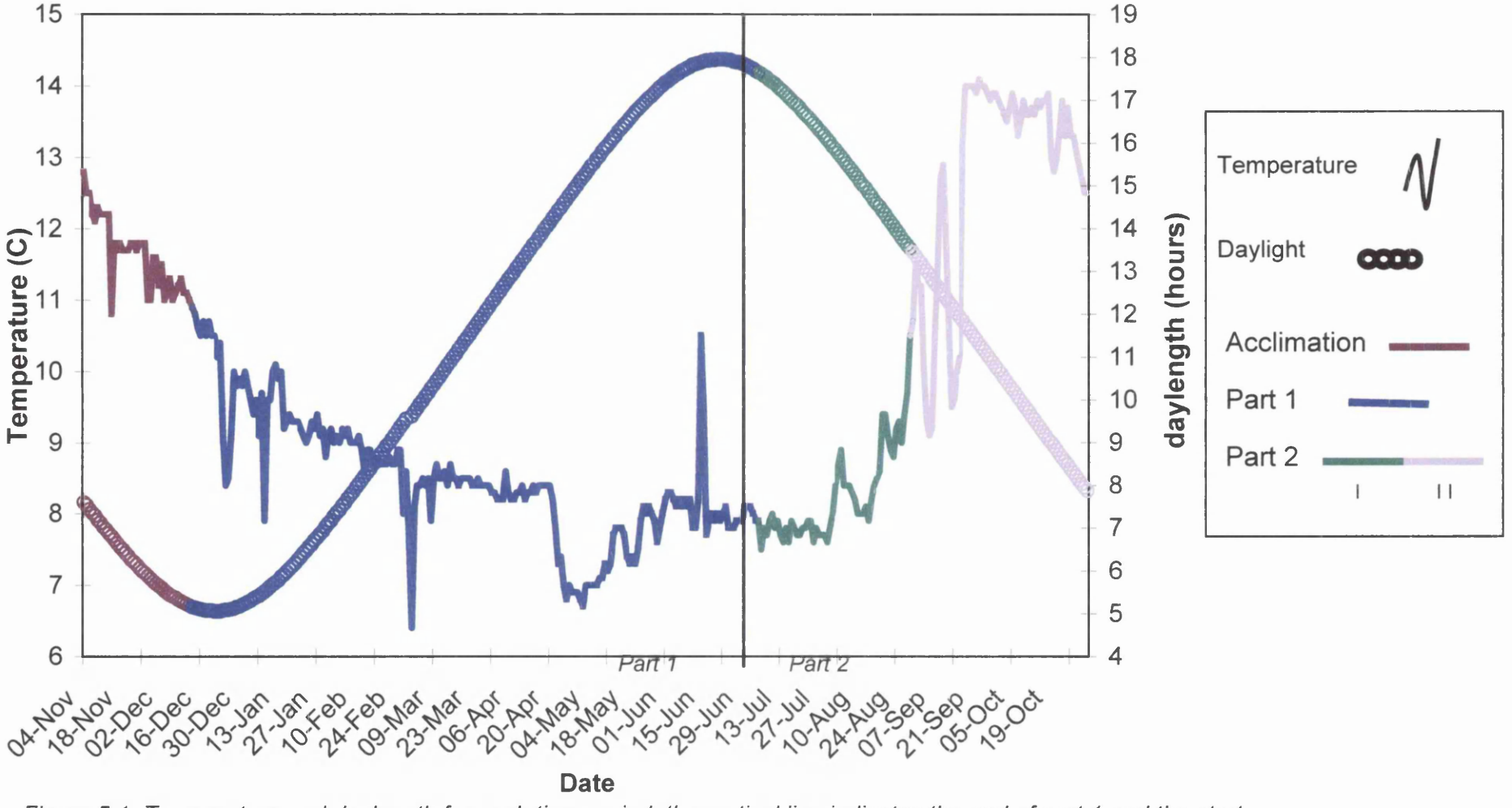


Figure 5.1 Temperature and daylength for each time period, the vertical line indicates the end of part 1 and the start of part 2

5. 2. 2 Part 1. Differential growth

5. 2. 2. 1 Subjects

In November 1999, 1200 fish from the same stock, weighing 1.34 kg ± 3%, were selected from 15m x 15m x 15m deep sea cages. They were placed in 10 circular experimental tanks, giving a density of 15.16kg.m^{-3}; commercial densities for Atlantic salmon are usually in the range of 12-15kg.m^{-3}. After four weeks of acclimation, 50% of the subjects were removed from each tank and fitted with VI tags (Northwest Marine Technology Ltd.) implanted in the post-ocular eyelid tissue (Niva, 1995). N.B. During weeks 1 to 13 of part 1, the three tanks fed at commercial ration levels were fitted with feed collectors, allowing waste feed to be monitored. These were removed after 100 days, as there was a significant increase in the incidence of injuries to the skin of fish in these tanks (chi-square test of association: $\chi^2 = 65.198$, d.f. = 1, $P < 0.001$).

5. 2. 2. 2 Feeding regime

Following a further 2 weeks of acclimation, the fish were exposed to experimental feeding regimes for a period of 27 weeks (beginning in January 2000). Commercial feed (EWOS Dynamic Red, size L) was used in all experimental tanks; two tanks were fed on a maintenance ration (0.15% body weight per day) and three tanks were fed on commercial feeding regimes (0.50% body weight per day). A further three tanks were fed on levels between maintenance and commercial feeding levels (0.25%, 0.35% and 0.45% body weight per day), and the remaining two tanks were fed to excess (0.6% body weight per day). For the purpose of analysis, the maintenance and low feeding regimes were termed 'low' and the commercial and excess feeding regimes were termed 'high'.

5. 2. 2. 3 Sampling and estimation of food intake

Fish were sampled three times over a four-day period, after 13, 21 and 29 weeks. Prior to sampling, commercial feed was replaced with feed labelled with X-ray opaque

glass beads (Ballotini grade 8.5: 400-450μm diameter Jencons Ltd., U.K.). The fish were exposed to experimental feeding regimes for 4 hours during the first two samplings, but this was increased to 6 hours during the third sampling in order to reduce the number of individuals with no record of feeding. Individuals were anaesthetised (Aqui-S – New Zealand), weighed, measured, identified and tagged individuals were X-rayed.

X-rays were taken using a Philips Practix, type XB 1021/00 machine at a distance of 1m from the fish, and the number of ballotini beads in the gastrointestinal tract of each fish was recorded from the developed X-rays. Each pellet contained 10 ballotini beads on average. Calibration of the number of ballotini beads per given weight of feed (R^2 = 0.92, $F_{1,89}$ = 1012.72, $P < 0.001$) provided the equation with which the amount of food eaten was calculated. During the study, seven fish were removed from the experiment and killed as they had sustained severe skin injuries, five were from experimental tanks containing feed collectors and two were from one of the tanks on the maintenance ration. These fish were omitted from all analyses.

Additionally, minor injuries were recorded, as these may have influenced growth or condition. The incidence of skin damage (exacerbated by abrasion against feed collectors in the three tanks on commercial feeding levels; present during the first 13 weeks of the experiment) and eye damage (believed to have been caused by abrasion against small screws on the tank windows that were present in all tanks throughout the experiment) was recorded. Skin damage was located on the sides and ventral surface of the fish and consisted of mild scale or small abrasive sores, where the scales were missing and flesh could be seen. Eye damage consisted of a slightly opaque film covering the eye and was usually only present on one eye. Six fish exhibited external signs of maturation and were also removed from the trial.

5. 2. 2. 4 Non-growing fish used in part 2

In order to create a population of previously non-growing fish, 106 fish of less than or equal to 1.34 kg, (the initial starting weight) were selected for part 2. The subjects were taken from two tanks on maintenance rations, one tank on low feeding regime, three tanks fed at commercial feeding levels and one tank fed to excess. Figure 5.2 summarises the selection methods used for this study.

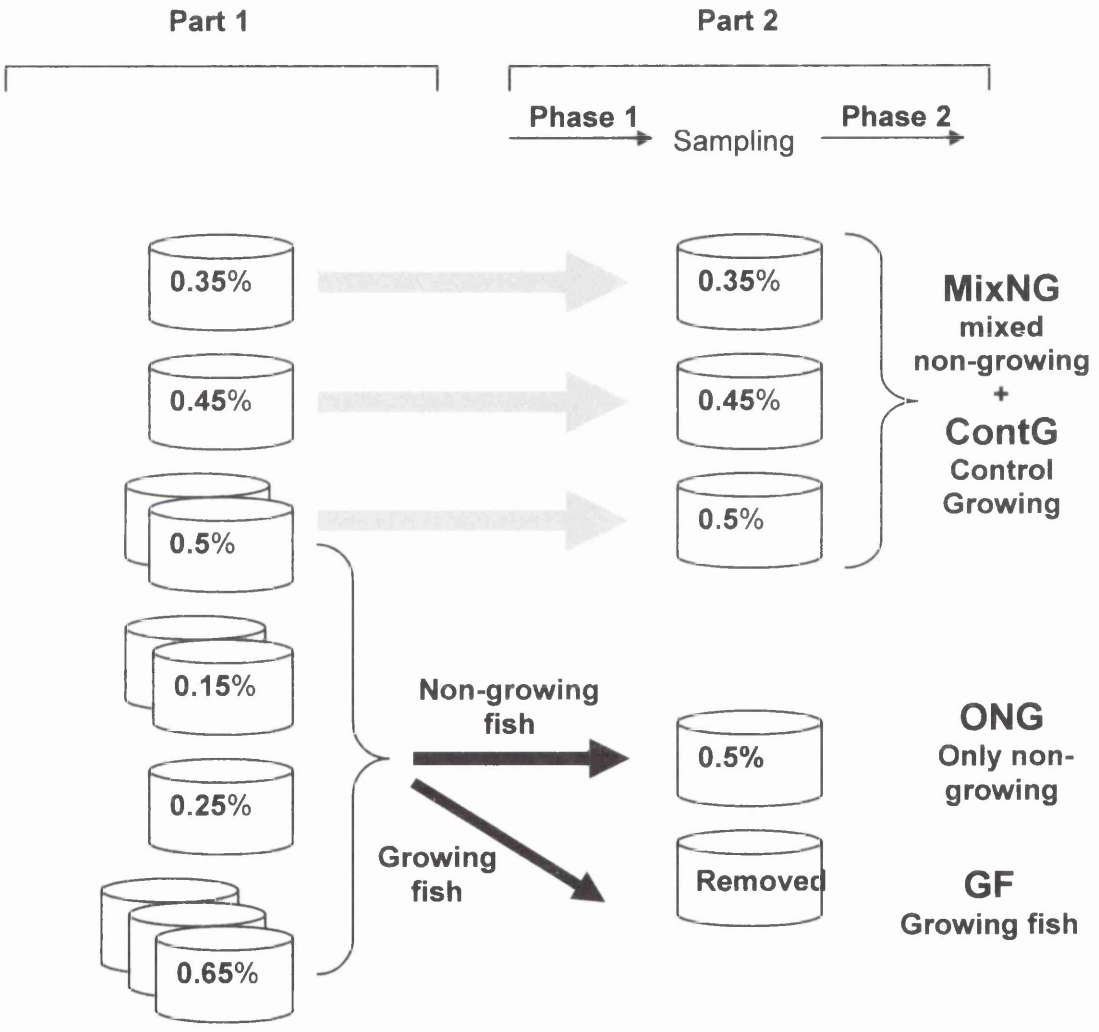


Figure 5.2 Summary of methods.

The remaining tanks (those fed on 0.35%, 0.45% and 0.6% bwpd) were left intact for a study investigating behavioural differences in fish of different sizes (Chapter 3). However, the individuals in these tanks provided a group of non-growing fish that were reared in the presence of large fish that could be used as a comparison for the 'only

non-growing' fish group (see below). In order to carry out these analyses the data from the three tanks of comparison fish were combined and then divided into subjects that had not grown during part 1 and those that had grown during part 1. This provided 28 non-growing fish and 277 growing fish.

5. 2. 3 Terminology

To aid clarity, the two groups fish that did not grow in part 1 were categorised as non-mixed non-growing (MixNG) and only non-growing (ONG) on account of the treatment that they were exposed to in part 2. The fish that did grow during part 1 in the tanks that provided the ONG (only non-growing) provided an exact control during part 1 for the non-growing fish and were referred to as growing fish (GF). However, the fish that did grow in the tanks that provided the MixNG (mixed non-growing) fish provided a control throughout parts 1 and 2 and were therefore referred to as control growing (ContG). These acronyms were used to refer to these fish during part 1 and part 2. See also figure 5.2

5. 2. 4 Part 2. Effects of social interactions and season

The population of ONG fish consisted of 25 non-growing fish exposed to high feeding regimes and 81 such individuals exposed to low feeding regimes. The selected fish were housed in one tank at a density of 11.01kg.m^{-3} and fed at a level recommended by commercial feed tables, of 0.5% body mass per day for 17 weeks. The subjects were not screened for feed intake during part 2, but were anaesthetised, identified, weighed, measured and checked for injuries during weeks 8 and 17. 43 non-growing fish retained VI tags from part 1, enabling the calculation of growth rates for two periods, namely the first 8 weeks and the remaining 9 weeks (referred to as phase I and phase II respectively), for these individuals.

The three populations of comparison fish were not exact controls for non-growing fish, as they were fed on different feeding regimes from ONG fish throughout part 1 and part

2 (0.35%, 045% and 0.6% bwpd). They were however grown in the same conditions, at the same time of year, and sampled at the same time as the ONG fish.

5. 2. 5 Growth and Condition

Growth was calculated using Thermal Growth Coefficient according to the following formula (Cho, 1992):

$$TGC = ((W_{t1}^{1/3} - W_{t0}^{1/3}) / T \times D)1000$$

Where W_{t0} represents weight in grams at time 0 and W_{t1} weight in grams at time 1, T, mean temperature in °C, and D, number of growth days.

A relative condition score for each fish was calculated by plotting a regression of weight upon length. Fish of a similar size to the ONG fish selected for part 2 were used to create the regression line. To do this, data from all fish during tagging, and GF fish exposed to commercial feeding regimes (0.5% bwpd) from each sampling in part 1 was used. The regression line of ln (weight) on ln (length) was plotted ($F_{1,179}$ = 10666.23, R^2 = 0.90, $P < 0.001$) and the condition for each fish was defined as the residual weight, for a given length, from this regression line (i.e. the difference between the observed and the expected weight). Thus, fish with heavier weights than expected had a positive condition, while those with lower weights than expected had a negative condition. Change in condition was calculated by subtracting condition at one sampling from condition at the previous sampling.

5. 2. 6 Statistical analysis

To compare the development of non-growing groups with those of growing groups, the performance of ONG and ContG fish was compared. In addition, the effect of release from conditions of social interaction on performance was investigated by comparing ONG and MixNG fish

Parametric tests were carried out where data sets displayed normal distributions and similar variances. However, the distribution of the weight data was not normal, and could not be transformed to be, so non-parametric Mann-Whitney U and Friedman tests were used for weight analysis. Repeated measures analysis of variance (ANOVA) tests were used to test the null hypotheses that there was no difference in the length or condition between GF fish and ContG fish (the two groups of growing fish) and between ONG and NG(P) fish (the two groups of non-growing fish) in part 1.

In part 2 repeated measures ANOVA tests were also used to test the hypotheses that there was no difference in the condition and length of ONG fish with and without eye damage. Repeated measures ANOVA tests were carried out using only tagged fish that were recorded during all samplings being tested. This resulted in the following sample sizes: ONG = 31, ONG with eye damage = 7, MixNG = 11, GF = 220, ContG = 108. The null hypothesis that there was no difference in the incidence of eating between ONG fish and GF fish was tested using the Mantel-Haenszel Procedure (Sokal & Rohlf 2000).

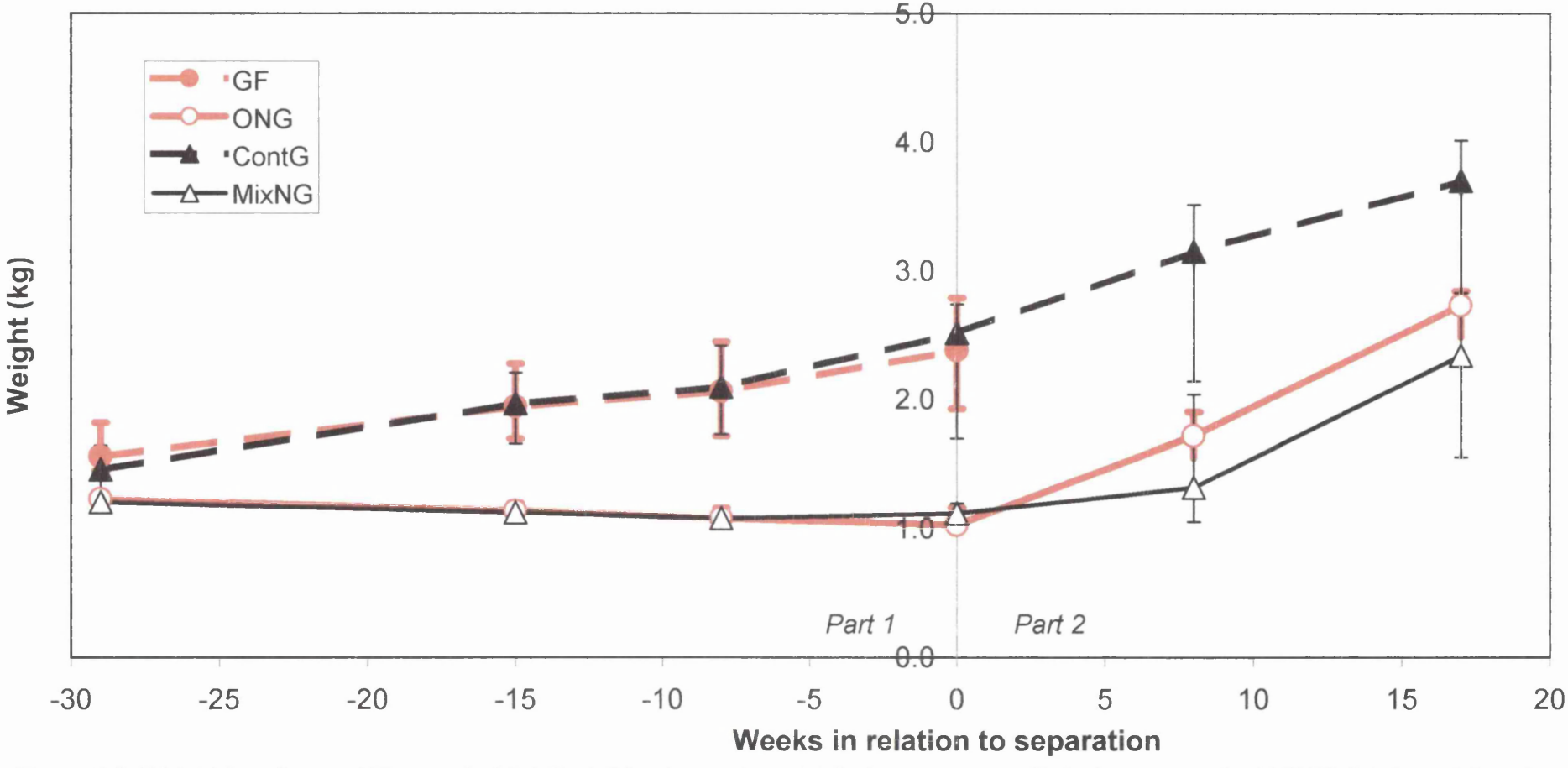


Figure 5.3 Weight (median and IQ range) of fish that did not grow in part 1 that were grown with 'only non-growing' (ONG) fish in part 2 and fish that were grown with larger fish; 'mixed non-growing' (MixNG) in part 2. Also presented are fish that did grow during part 1; 'growing fish' (GF) that were in the same tanks as the ONG fish and 'control growing' fish (ContG), that were grown in the same tanks as the MixNG fish.

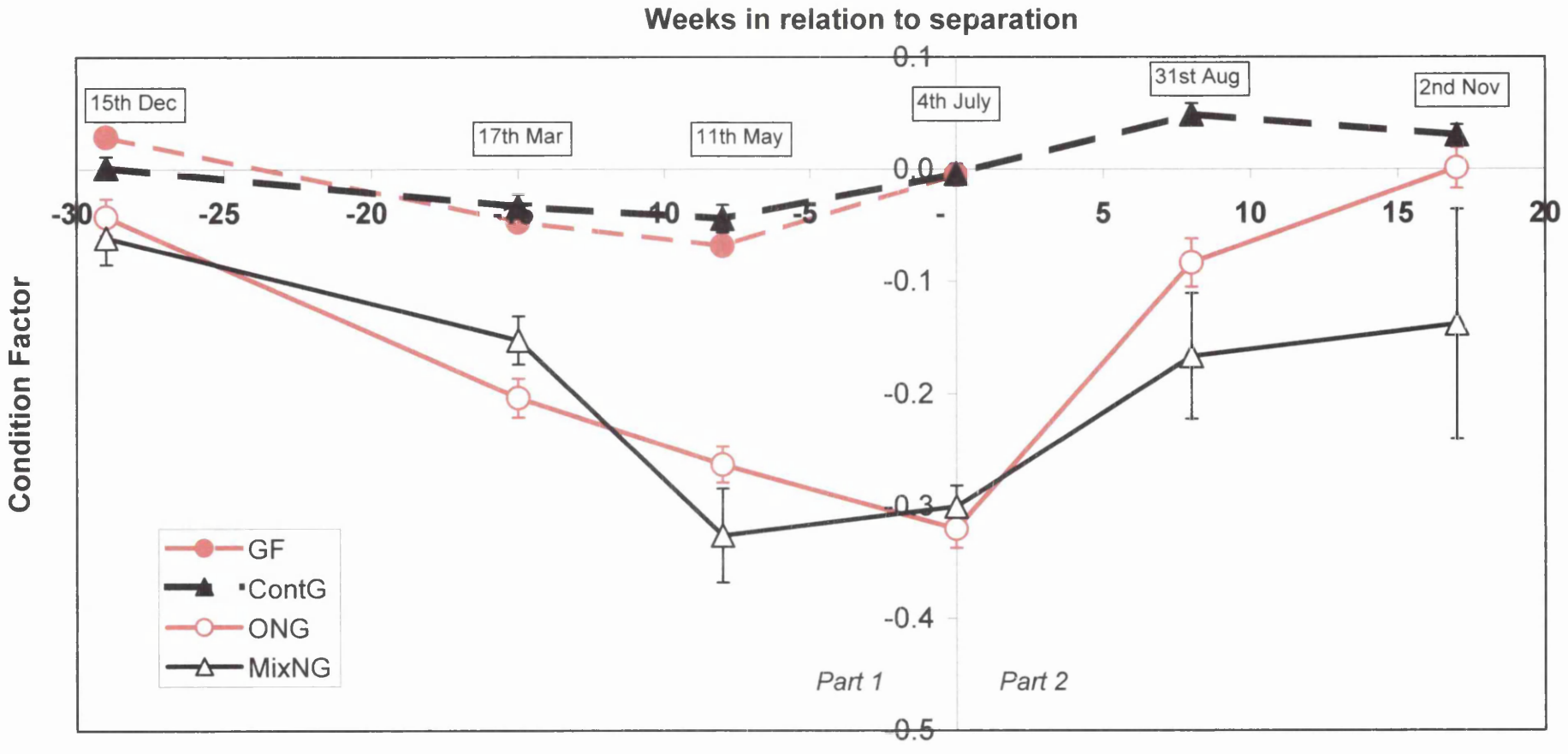


Figure 5.4 Condition (mean and SEM) of fish that did not grow in part 1 that were grown with 'only non-growing' (ONG) fish in part 2 and fish that were grown with larger fish; 'mixed non-growing' (MixNG) in part 2. Also presented are fish that did grow during part 1; 'growing fish' (GF) that were in the same tanks as the ONG fish and 'control growing' fish (ContG), that were grown in the same tanks as the MixNG fish.

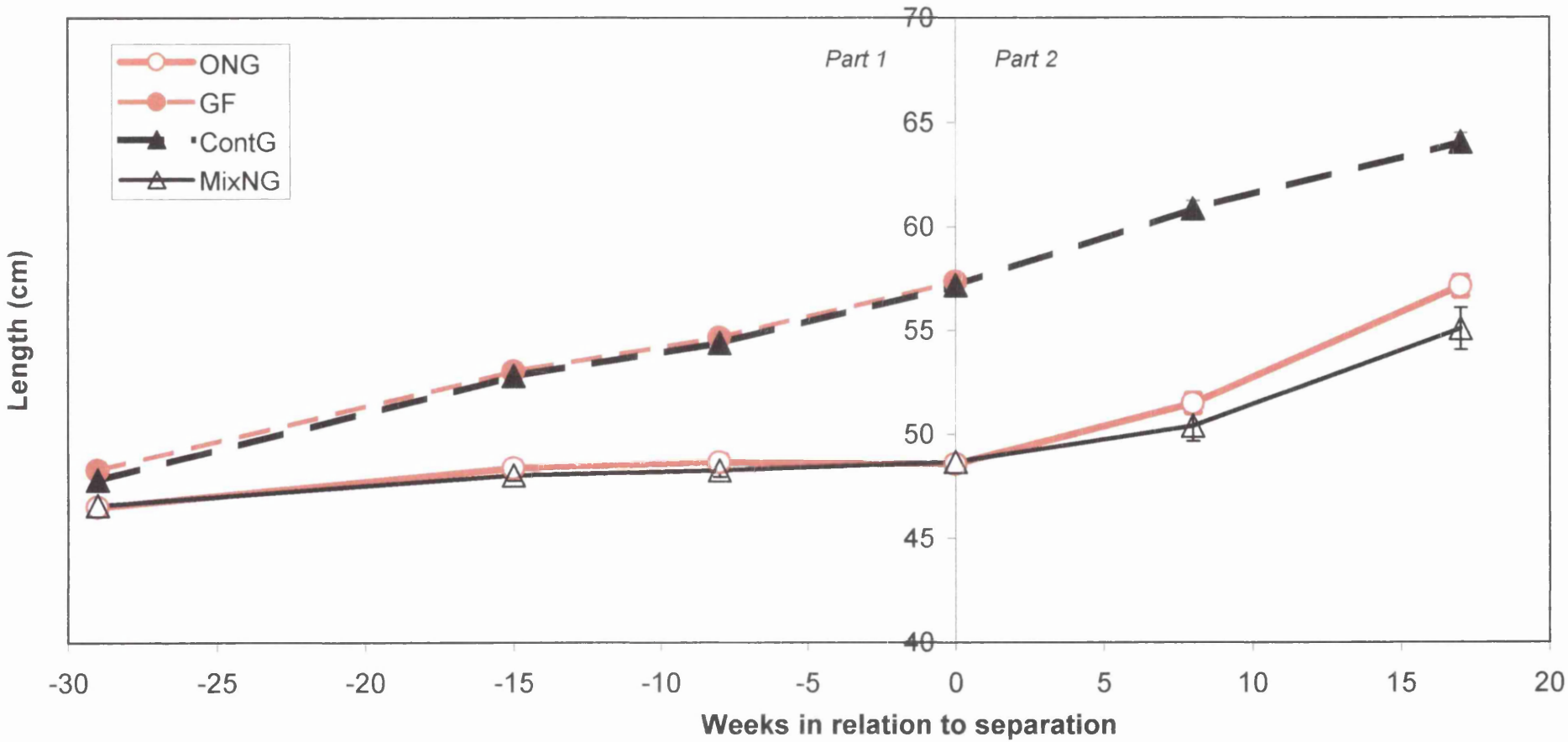


Figure 5.5 Length (mean and SEM) of fish that did not grow in part 1 that were grown with 'only non-growing' (ONG) fish in part 2 and fish that were grown with larger fish; 'mixed non-growing' (MixNG) in part 2. Also presented are fish that did grow during part 1; 'growing fish' (GF) that were in the same tanks as the ONG fish and 'control growing' fish (ContG), that were grown in the same tanks as the MixNG fish.

5. 2. 7 Time of placement in 'only non-growing' (ONG) fish tank

There was a significant effect of day of placement (in ONG tank) on growth for the first 8 weeks (one-way ANOVA; $F_{3,35} = 2.91$, $P = 0.048$); fish that arrived on day one had higher growth rates. However, this effect was heavily influenced by subjects with eye damage, as removal of such fish from the analysis also removed the effect of arrival day (one-way ANOVA: $F_{3,34} = 0.655$, $P = 0.587$). There was no effect of day of arrival on growth after 17 weeks: $F_{3,35} = 0.316$, $P = 0.814$), nor was there was there any effect of arrival day at the start of part 2 on weight after 8 or 17 weeks (8 weeks: one-way ANOVA: $F_{3,38} = 2.218$, $P = 0.103$; 17 weeks: $F_{3,38} = 1.566$, $P = 0.215$).

5. 3 RESULTS

5. 3. 1 Performance during part 1

During part 1, there was an initial difference in the weight of GF fish and ContG fish; GF fish were smaller than ContG fish (Figure 5.3; Mann-Whitney U = 11816.00, Z = -2.498, $P = 0.013$). No difference was found in subsequent samplings during part 1 (Figure 5.3; Mann-Whitney U, 13 weeks, U = 13471.00, Z = -0.687, $P = 0.492$, 21 weeks, U = 14535.50, Z = -0.528, $P = 0.597$, 29 weeks: U = 14724.00, Z = -1.439, $P = 0.150$). Furthermore, there was no overall difference in the condition or the length of fish from the two categories during part 1 (repeated measures ANOVA, condition: Figure 5.4, $F_{1,326} = 0.166$, $P = 0.281$; length: Figure 5.5; $F_{1,326} = 0.030$, $P = 0.863$).

Similarly, there were no differences in the weight of the two groups of non growing fish during part 1; ONG fish and NG(P) fish (Figure 5.3; Mann-Whitney U, Initially: U = 211.50, Z = -0.743, $P = 0.457$; 13 weeks: U = 237.50, Z = -0.011, $P = 0.991$; 21 weeks: U = 218.00, Z = -0.071, $P = 0.943$, 29 weeks: U = 176.00, Z = -1.530, $P = 0.126$). Nor was there a difference between the condition or length of the ONG fish and NG(P) fish

(repeated measures ANOVA: between subjects effects, condition, $F_{1,44} = 0.175$, $P = 0.677$; length, $F_{1,44} = 0.175$, $P = 0.677$).

The number of fish with injuries at the end of part 1 was not affected by previous regime ($\chi^2 = 1.044$, d.f. = 1, $P = 0.330$); 30% of ONG fish had minor injuries; 12% had skin damage, 17% had eye damage and 8% had both. There was no effect of eye damage on weight, length or condition of fish at the start of part 2 (Mann-Whitney U test: weight: U = 138.000, Z = -0.063, $P = 0.963$; condition: U = 88.500, Z = -1.524, $P = 0.130$, length; U = 91.000, Z = -1.537, $P = 0.132$).

5. 3. 2 Injuries and growth in 'only non-growing' (ONG) fish

Initially, fish that had not grown in part 1 had a greater incidence of skin injuries than eye injuries. The incidence of both kinds of injury, but particularly of sores, decreased during part 2, indicating that healing was taking place (Figure 5.6). Examination of the effect of injury at the start of part 2 revealed no effect of initial skin damage on growth during part 2, in terms of weight, condition or length (repeated measures ANOVA: between subjects effects, TGC, $F_{1,37} = 0.012$, $P = 0.913$; change in condition, $F_{1,37} = 0.016$, $P = 0.901$; change in length, $F_{1,37} = 0.071$, $P = 0.792$). However, initial eye damage did affect performance in part 2. There was a difference in the weight increase of individuals with and without eye injuries (Table 5.1; repeated measures ANOVA: between subjects effects, $F_{1,37} = 330.982$ $P < 0.001$); fish with eye injuries had lower growth. In terms of increase in condition, there was no difference between fish with eye injuries and fish without eye injuries (Table 5.1; repeated measures ANOVA, between subjects effects $F_{1,37} = 0.384$, $P = 0.539$), but there was a significant interaction between time period and injury status (repeated measures ANOVA, within subjects effect $F_{1,37} = 5.158$, $P = 0.029$), indicating that fish with eye injuries had a much lower increase in phase I, but a much greater increase in phase II. Individuals with eye damage were removed from all further analyses.

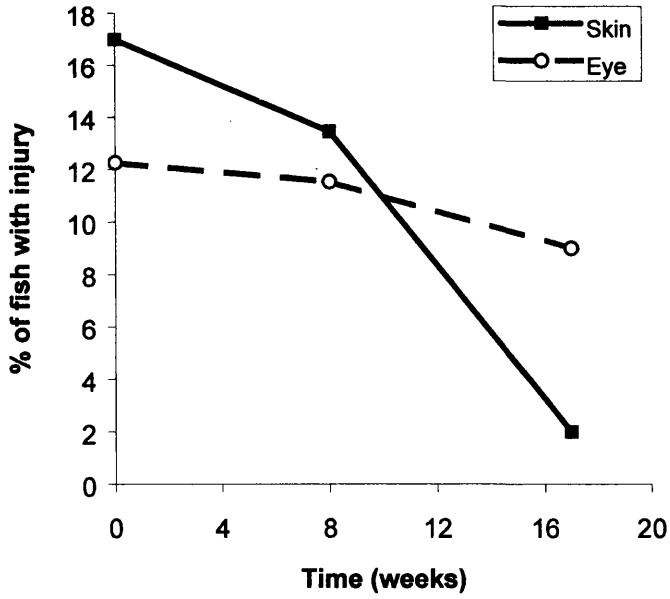


Figure 5.5 Incidence of skin and eye injuries in ONG (non-growing) fish during part 2

Table 5.1 Summary of weight (median, interquartile range), condition (mean ± SEM), and length (mean ± SEM) of 'only non-growing' (ONG) fish with and without eye injuries during part 2.

	Mean ± SEM / Median,IQ range		Result from Mann-Whitney U test		
	NGA	GF	U	Z	*P*
Weight (kg)	1.23,0.083	1.56,0.37	2174.5	-7.118	*< 0.001*
Condition score	-0.043 ± 0.016	0.029 ± 0.006	4180.00	-3.962	*< 0.001*
Length (cm)	46.5 ± 0.22	48.3 ± 0.10	3019.50	-5.817	*< 0.001*

5. 3. 3 Initial condition of 'growing fish' (GF) and 'only non-growing' (ONG) fish

Although the subjects for this study were initially tightly graded, they were then fed *ad lib* for one month, during acclimation. At this point, in mid-December, ONG fish were already different from GF fish (Mann-Whitney U test). ONG fish were smaller in weight and length and in addition, had a lower condition than GF fish (Table 5.2).

Table 5.2 Summary of initial weight, condition and length of ONG (non-growing) and GF (growing fish), during part 1, and the results from Mann-Whitney U tests comparing ONG with GF fish.

	Mean ± SEM / Median,IQ range		Result from Mann-Whitney U test		
	ONG	GF	U	Z	*P*
Weight (kg)	1.23,0.083	1.56,0.37	2174.5	-7.118	*< 0.001*
Condition score	-0.043 ± 0.016	0.029 ± 0.006	4180.00	-3.962	*< 0.001*
Length (cm)	46.5 ± 0.22	48.3 ± 0.10	3019.50	-5.817	*< 0.001*

5. 3. 4 Part 1: Performance of 'growing fish' (GF) and 'mixed non-growing' (MixNG) fish

The selection criterion for part 2 was a weight equal to or less than the starting weight (1.34kg), however, some ONG fish had significantly decreased in weight during part 1 (Wilcoxon signed ranks test: Z = -3.038, P = 0.002). As GF fish increased in weight (Figure 5.3; Friedman test: χ^2 = 75.577, d.f. = 3, P <0.001), the difference between the weight of ONG fish and GF fish increased during part one (Figure 5.3; Mann-Whitney: 13 weeks, U = 757.00, Z = -8.269, P < 0.001; 21 weeks, U = 613.50, Z = -8.669, P < 0.001; 29 weeks, U = 407.00, Z = -9.435, P < 0.001).

The condition of both ONG and GF fish decreased during part one (Figure 5.4; repeated measures ANOVA: within subjects effects, $F_{3,250}$ = 110.477, P < 0.001; linear term of polynomial contrasts: $F_{1,252}$ = 226.571, P < 0.001). ONG fish had a lower condition than GF fish (Figure 5.4; repeated measures ANOVA: between subjects effects, $F_{1,252}$ = 174.225, P < 0.001) and the significant interaction between fish category and time indicated that the condition of ONG fish decreased to a greater extent over time (Figure 5.4; repeated measures ANOVA: within subjects effects, $F_{1,250}$ = 24.572, P < 0.001).

Examination of length over time showed that the decrease in condition in ONG fish was in part due to a relative increase in length, not just a decrease in weight. All fish increased in length over time (Figure 5.5; repeated measures ANOVA: within subjects effects, $F_{3,250} = 197.438$, $P < 0.001$; linear term of polynomial contrasts, $F_{1,252} = 515.289$, $P < 0.001$), but ONG fish were shorter in length than GF fish (Figure 5.5; repeated measures ANOVA: between subjects effects, $F_{1,252} = 173.836$, $P < 0.001$). A significant interaction between fish category and time indicated that this relationship changed over time (Figure 5.5; repeated measures ANOVA: within subjects effects, $F_{3,250} = 55.676$, $P < 0.001$); ONG fish increased in length to a lesser extent and therefore the difference between the length of GF and ONG fish increased over time.

Fewer ONG fish than GF fish ate (Table 5.3) at each sampling point in part 1 (Mantel-Haenszel procedure: $\chi^2 = 2.008$, d.f. = 1 $P = 0.176$). Combining all samplings, GF fish were 3.4 times more likely to have eaten than ONG fish. (Mantel-Haenszel procedure: $\chi^2 = 17.689$, d.f. = 1, $P < 0.01$). Comparison of the amount eaten between GF fish and ONG fish was not possible due to the large number of zero counts of feed intake. Throughout part 1 there was no difference in the proportion of injured fish in those that grew and those that did not (see Table 5.3).

Table 5.3 The percentage of 'growing fish' (GF) and 'only non-growing' (ONG) fish that ate during part 1 at 13, 21 and 29 weeks.

proportion of fish eating	Sampling (week) 11	19	27
ONG fish	17%	11%	31%
GF fish	27%	41%	61%

5. 3. 5 Part 2: Performance of 'only non-growing' (ONG) fish and 'mixed non-growing' (MixNG)fish

In the described study, the ONG fish grew in the absence of larger fish, when they were reared with similar 'only non-growing' fish. In phase I, 8% of ONG fish did not grow, but all ONG fish grew in the second. There was a significant increase in weight between the start and end of phase I (Wilcoxon signed ranks, Z = -4.995, $P < 0.001$; Figure 5.3) and also between the start and end of phase II (Wilcoxon signed ranks, Z = -4.861, $P < 0.001$; Figure 5.3). However, MixNG fish also grew and there was no difference in the weight of ONG and MixNG fish at the end of phase I or phase II of part 2 (Mann Whitney U test: phase I, U = 151.50, Z = -1.194, $P = 0.236$; phase II, U = 136.00, Z = -1.354, $P = 0.183$). In addition, an independent samples t-test revealed that there was no difference in the growth rates of ONG fish and MixNG fish in phase I of part 2 (mean ± SEM (TGC): ONG fish = 3.40 ± 0.248, MixNG fish = 2.31 ± 0.734; $t_{12.4} = 1.410$, $P = 0.183$). Nor was there a difference in the growth of ONG and GF fish in phase I of part 2 (t - test: $t_{37} = -1.402$, $P = 0.169$).

The condition of the ONG fish and the MixNG fish was very low at the start of part 2, but then increased. Repeated measures ANOVA tests revealed a significant change in condition of both ONG and MixNG fish during part 2 (within–subjects effects used as Mauchly's test of sphericity was non-significant, $F_{2,80} = 41.338$, $P < 0.001$). This change represented a significant increase in condition (linear term of polynomial contrasts: $F_{1,40} = 62.007$, $P < 0.001$). Overall, there was no difference in the condition

of ONG and MixNG fish during part 2 ($F_{1,40}$ = 1.822, P = 0.185), but there was an interaction effect between fish category (ONG or MixNG) and time ($F_{2,40}$ = 3.623, P = 0.031) indicating that ONG fish had a sharper increase in condition than MixNG fish during part 2. There was no difference in the final condition of ONG and MixNG fish (mean ± SEM: ONG = -0.0003 ± 0.018, MixNG = -0.1381 ± 0.102; independent samples t-test: t_{12}, = 1.33, P = 0.208).

Similarly, there was a significant increase in length of both ONG and MixNG fish in part 2 (repeated measures ANOVA, tests of within subjects used, $F_{2,80}$ = 298.770, P < 0.001; linear term of polynomial contrasts, $F_{1,40}$ = 398.468, P < 0.001) and there was no difference in the increase shown in ONG and MixNG fish (between subjects effects, $F_{1,40}$ = 1.262, P = 0.268). Nor was there any interaction between time and fish category ($F_{2,80}$ = 2.726, P = 0.072).

There was a difference in the change in length between fish with eye damage and those without (repeated measures ANOVA: between subjects effects, $F_{1,37}$ = 24.744, P < 0.001); fish without eye damage increased in length to a greater extent than those with eye damage. Due to the differences in performance of fish with eye injuries and fish without, those with eye injuries were removed from the main analyses in part 2.

5. 4 DISCUSSION

5. 4. 1 Effect of season on performance of non-growing fish

The described study demonstrated that fish that had not grown for 29 weeks were capable of growth. This was apparent both in fish that were separated from larger fish and in those that remained in populations with larger fish. In addition, although the ONG fish lost condition during the period of non-growth, they recovered, and indeed

increased their condition above their initial state to a level similar to that of the ContG fish.

Both groups of non-growing fish exhibited a dramatic increase weight, length and condition during part 2, which suggests the influence of an environmental trigger on the growth of previously non-growing fish. Seasonal variation in growth, condition and feeding rates are well documented in wild juvenile Atlantic salmon (e.g. Gardiner & Geddes 1980). However, circannual rhythms in growth (Forsberg, 1996), condition (Kadri, *et al.,* 1996b) and feeding (Jørgensen & Jobling, 1992; Blyth *et al.,* 1999) have also been observed in Atlantic salmon held in commercial conditions under a natural photoperiod. These studies indicated that Atlantic salmon underwent a decrease in growth and condition over winter. In the described study, individuals that had not grown and therefore had a relatively lower weight, condition and length underwent a marked increase in each of these characteristics in autumn. These fish may have been responding to an environmental trigger that stimulated them to grow and increase condition to enable them to survive over winter.

Throughout the trial, the subjects were grown in tanks under a constant light regime. Nevertheless, the small circular window in the side of the tank may have enabled the fish to pick up some seasonal cues. Additionally, although the water source was at a depth of 30m, there was seasonal variation in the water temperature of the tanks. Natural endogenous rhythms can persist even whilst animals are held in completely different conditions; Eriksson (1978) and Sæther *et al.*, (1996) demonstrated that circannual rhythms occur in brown trout and Arctic charr held under a constant photoperiod (of 12:12 LD). Therefore, it is likely that the growth of the fish in the described study was affected by either endogenous or exogenous circannual rhythms.

5. 4. 2 Effect of social interaction on performance of 'only non-growing' (ONG) fish

Although there was no difference between the growth rates of ONG fish during part 1 and ContG fish during phase I of part 2, the fact that the non-growing fish underwent a rapid increase in condition indicates that they were undergoing some form of compensatory growth. Several authors have suggested that compensatory growth in salmonids is triggered by an individuals' assessment of its ratio of fat stores to structural tissue (Jobling & Miglavs, 1993; Broekhuizen *et al.*, 1994; Johansen *et al.*, 2001). In the present study, individuals that did not grow during part 1 may have decreased their fat stores to a critical level by the end of part 1 (as they had not grown for 29 weeks), thus triggering the observed increase in part 2. However, as part 2 began around the time of the summer solstice, a strong environmental cue, it is possible that this triggered the observed increase in weight, length and condition (see MacLean & Metcalfe 2001).Previous studies have shown that compensatory growth occurs after a period of food deprivation (Jobling & Koskela, 1996; Morgan & Metcalfe, 2001) and low temperatures (Mortensen & Damsgård, 1993; Nicieza & Metcalfe, 1997; MacLean & Metcalfe, 2001). The described study presented evidence of compensatory growth occurring after a period of behaviourally induced growth suppression.

This study does not clearly show if restricted access to feed prevented the ONG fish from growing, but ONG fish were less likely to have eaten than GF fish. Stressed individuals have been shown to have a lower Food Conversion Ratio (Jobling & Wandsvik, 1983; Abbott & Dill, 1989), so the absence of growth in these individuals may be partly due to inhibition of physiological processes such as feed assimilation. However, as the proportion of ONG fish that had eaten was lower than the proportion of GF fish, it is likely that ONG fish may have fed less often than individuals that did grow. Previous studies have shown that smaller, subordinate fish have a higher variation in their daily food intake than large fish (Kristiansen, 1999; Carter *et al.*, 1992;

McCarthy *et al.* 1992a), and feed opportunistically at different times of the day from others (Kadri *et al.*, 1997).

5. 4. 3 Development of 'only non-growing' (ONG) fish

Although all fish were initially tightly size-graded from the sea-cage into the experimental tanks, the ONG fish were smaller in weight and length and had a lower condition after only 4 weeks in these tanks. Feeding hierarchies have been documented in Atlantic salmon in commercial conditions (Thorpe *et al.*, 1990) and in Arctic charr in large groups (Øverli *et al.*, 1999a). During the formation of such hierarchies, the failure of an individual to ensure a good position within the hierarchy could affect the future growth of that individual. Although the subjects were initially tightly graded into the experimental tanks from the sea-cages, this was carried out on the basis of weight, not condition or length. Therefore, the ONG fish may have differed in length or condition from the GF fish during selection for the study. Some authors have noted the presence of stepwise growth in salmonids, with periods of synthesis and growth followed by periods of apparent quiescence (Wagner & McKeown, 1985). The ONG fish may have been undergoing a period of non-growth during transfer and therefore were not part of the initial formation of the feeding hierarchy. Alternatively, the ONG fish may have taken longer to acclimatise to the experimental tanks, resulting in their initial exclusion from the hierarchy.

Perhaps surprisingly, although the ONG fish decreased in weight throughout part 1, they increased in length. Maintenance costs are minimised during periods of low rations (Boujard, 2000; Russell & Wooton, 1992), but deposition does still occur (e.g. Miglavs & Jobling, 1989). Lie and Huse (1992) and Einen *et al.*, (1998) both noted an increase in length during weight loss in starved fish. Body mass may be energetically easier and quicker to build up than skeletal mass (see also Nicieza & Metcalfe, 1997), as salmonids have been observed to show a greater relative increase in bodymass than length when feed levels increase after a period of restriction (e.g. rainbow trout,

Weatherley and Gill, 1981; Atlantic salmon, Nicieza & Metcalfe, 1997). Therefore, fish may preferentially allocate resources to skeletal growth under extreme conditions.

Alternatively, the observed increase in length of the ONG fish may have been a result of environmental cues and related to season. Mørkøre and Rørvik (2001) noted in Atlantic salmon in sea-cages, that there was a greater increase in length than body mass during late autumn and winter. The length increase shown by the ONG fish in this study occurred from December until July, but it may have been triggered by lower temperatures throughout this time.

5. 4. 4 Effect of injury on performance of 'only non-growing' (ONG) fish

Fish that had not grown in the differential growth period had a higher incidence of injuries than individuals that had grown. ONG fish therefore either sustained a larger number of injuries, or had a reduced ability to heal than fish that did grow. These injuries may have occurred during competition for food, because of direct aggression from other individuals, or from abrasion against objects in the experimental tanks. Individuals competing for food may sustain injuries inadvertently under conditions of intense competition, for example when many individuals compete frantically for feed. Some of the injuries may have resulted from abrasion against feed collectors and window attachments. If this were the case, the persistence of these injuries suggests either an increased intensity of initial injury or a reduced ability to recover from an injury. Stressors such as disease or inadequate nutrition can reduce the amount of energy available for healing (Schreck, 1981). The ONG fish in this study may have been severely nutritionally stressed, due to restricted access to food. In addition, ONG fish in low feeding regimes had a higher incidence of injury than those from high regimes, implying that levels of competition, and therefore stress were higher in low feeding regimes.

The proportion of ONG fish was greater in populations fed on low feeding regimes, indicating that competition for food increased the occurrence of ONG fish. In addition, non-growing individuals from high feeding regimes were smaller, suggesting that only the very poorest fish (in terms of competition or performance) did not grow in the high feeding regimes.

5. 4. 5 Compensatory growth in 'only non-growing' (ONG) fish

In part 2 the ONG fish did not fully compensate for their loss of growth in part 2; the weight of the ONG fish was still lower than that of the ContG fish. This may have been due to the timing of the end of the study (17 weeks after the ONG fish were removed from the GF fish); full compensation may have occurred over a longer time period. Alternatively, the extent of compensatory growth has been shown to correlate negatively with the extent of food deprivation. Individuals on low rations during the restriction period show incomplete compensatory growth (Boujard *et al.* 2000; Johansen *et al.* 2001) as complete weight compensation may depend on sufficient food intake to allow protein deposition (see Jobling *et al.*, 1993). Some of the ONG fish in this study may have been deprived of feed completely, as they lost weight. In addition, compensatory growth may have been slow at the start of part 2 as a result of low temperatures; Dobson and Holmes (1984) found low compensatory growth in rainbow trout below 9°C. During part 2, ONG fish increased initially in weight relatively more than in length (during phase I), this may have been due to the initial replenishment of white muscle or lipid reserves that were depleted during part 1 (see Black & Love, 1986; Morgan & Metcalfe 2001)

The ONG fish in this study did undergo full compensatory growth in terms of condition. Exactly what condition is measuring is not clear, but Einen *et al.* (1998) noted a correlation between the visual fatness score of gutted fish and condition during starvation. The apparent return to initial condition may indicate that energy stores were repleted.

5. 4. 6 Application of results

In the natural environment, smaller fish may employ a strategy that avoids competition and conserves energy, rather than directly competing for food (Metcalfe, 1986). As commercially reared fish retain features in common with their wild ancestors (Huntingford & Thorpe, 1990), they may express behavioural traits that evolved for survival in nature, but are detrimental to the aquaculture industry. Current commercial practice is to discard fish that are not growing under production conditions, but the ONG fish in part 1 of this study demonstrated the ability to grow. Similar results have been found in carp (Wohlfarth, 1977), sturgeon (Georgiadis *et al.*, 2000a), and prawns (Karplus *et al.*, 1992). Additionally, Georgiadis *et al.* (2000b) showed that the practice of separating poor-growing sturgeon from larger individuals and growing them was financially profitable. It may be economically beneficial to remove ONG fish and raise them separately, instead of discarding them. Currently, it is difficult to assess the viability of this practice as discarding of non-growing fish occurs at many points throughout production and is often un-recorded. Engineering rearing conditions that enhance natural feeding behaviours and strategies of all fish and not just the majority may increase production levels and decrease costs, both to the environment and to the farmer. For example, feeding regimes that account for all individuals within a population would decrease uneaten food and hence pollution and increase profits.

In juvenile wild salmonids, dominant individuals obtain the best territories and the greatest amount of food, under certain conditions at least (Fausch, 1984; Hughes, 1992; but see Bachman, 1984). Serial removal tests have been used in the laboratory to determine dominance status in small groups (Metcalfe *et al.*, 1989). In these tests the dominant individual is identified then removed to allow the next-dominant individual to be identified. These tests have suggested that animals can move up in the hierarchy upon the removal of the top individual.

This study presented no clear evidence that the removal of non-growing individuals from populations containing large, growing individuals could result in the rapid growth of the previously non-growing individuals. However, this result is not conclusive due to the absence of a control group grown under identical conditions. Further studies are necessary to determine the mechanisms behind the absence of growth in some individuals within large populations.

5. 4. 7 Conclusions and implications

The present study demonstrated that non-growing, presumably subordinate (see chapters 3 & 4), fish gradually loose weight and condition (but not length) during a 29 week period, up to the summer solstice. However, following this, these subordinate fish grow well. Those that are removed from social pressure show a more rapid increase in condition than those that remain with larger fish. The practical implications from this study are that grading may not be necessary in commercial aquaculture conditions.

CHAPTER 6. COMPETITIVE ABILITY AND NUTRITIONAL STATUS AS DETERMINANTS OF GROWTH IN ATLANTIC SALMON

6. 1 INTRODUCTION

Artificial selection has increased the growth rates of many species of farmed fish (Gjerde, 1986; Gross, 1998), but there is still a great deal of variation in the resultant growth rates. Due to the controlled conditions encountered in aquaculture, fundamental biological questions on intraspecific growth differences can be answered using farmed fish. Growth variation is also of interest to aquaculturists as it presents an opportunity to increase profits and decrease losses. In salmonid aquaculture, there is a need to reduce the number of poor growing fish of unacceptable size and quality. Aquaculturists are under pressure from customers to produce a high quality, uniform product. Growth heterogeneity of farmed salmonids is known to decrease with increasing ration levels (Jobling & Koskela, 1996; Damsgard *et al.*, 1997). However, feed costs currently make up a large proportion of production costs (40%, Juell *et al.*, 1994; 50-60%, Sveier & Lied, 1998) and are likely to increase due to the rising cost of fishmeal, a major fish feed constituent.

Individual variation in growth rates can be determined by persistent individual differences in the ability to acquire and / or process food. Growth variation increased in Arctic charr and juvenile cichlids, as a result of dominant individuals preventing subordinates from feeding (Jobling, 1985; Koebele *et al.*, 1985). However, subordinate Arctic charr had a lower rate of nutrient utilisation (Olsen & Ringø, 1999), and a more

efficient food conversion was observed in faster growing Medaka (Ruzzante & Doyle, 1993).

In contrast, Abbot and Dill (1989) attributed growth variation in subordinate and dominant juvenile steelhead trout to the influences of social stress on metabolism. Recently, several studies have confirmed the link between individual differences in performance and metabolism. A higher resting metabolic rate was reported in the better competitor (in terms of food intake and spatial position) in pairs of juvenile rainbow trout (McCarthy 2001), and increased growth variation was observed in groups of juvenile Atlantic salmon with higher metabolic rate (Cutts *et al.*, 1998).

Individual differences in growth rate may also be the result of differences in life history processes or reversible differences in body condition. Responses to current nutritional status can also affect growth in the short or mid term. For example, rainbow trout that have undergone the greatest degree of growth depensation during a period of food restriction show the greatest degree of compensatory growth (Jobling & Koskela, 1996; reviewed by Broekhuizen *et al.*, 1994). In addition, MacLean and Metcalfe (2001) suggested that compensatory growth could occur as a result of a deviation from the individual's target body size for a particular time of year.

In a previous experiment, populations of fish were grown under a variety of feeding intensities, ranging from maintenance to over-feeding (see chapter 2). This provided the opportunity to compare the performance of fish that had reached the same weight by either growing well in a poor environment (good performers), or by growing poorly in a rich environment (poor growers). The described study measures the performance of these fish when placed together in conditions of intermediate food supply. In addition, since fish of the same weight may differ in length, the effect of initial body condition on patterns of compensatory growth was investigated.

The aims of this study were to compare growth rates (in terms of weight, condition and length) in conditions of intermediate food supply in good and poor performing Atlantic salmon, and to relate the observed patterns of growth to nutritional status

6. 2 METHODS

This study was split into two parts: part 1 provided fish of similar sizes, but differing growth history; part 2 monitored the growth of selected good and poor performing fish from part 1. Part 2 consisted of a further two phases; phase I began on the 8th of July 2000 and ended on the 30th of August 2000 (8 weeks); phase II ran from this point until the 1st of November 2000 (9 weeks). The conditions during these two phases were distinct in terms of temperature, length of day and growth rates.

6. 2. 1 Experimental conditions

Throughout parts 1 and 2, the subjects were housed in circular, 2m deep x 3m diameter tanks. Seawater was supplied from a depth of 30m, providing an ambient water temperature. Water entered the tank through a vertical pipe at the side of the tank at 210 l.min^{-1} and exited from the centre of the tank base, giving a circular flow. The oxygen level in the outlet ranged from 75 to 80% saturation and the light regime was 12h:12h light:dark. The conditions for part 1 are outlined in detail in chapter 2.

6. 2. 2 Part 1: Differential growth

In November 1999, 1200 fish from the same hatchery stock, weighing 1.34 kg ± 3%, were selected from 15m x 15m x 15m sea cages. 10 populations of 120 fish were created and transferred into the experimental tanks, at a density of 15.16kg.m^{-3} (commercial densities for Atlantic salmon are in the range of 12-15kg.m^{-3}). After four weeks of acclimation, 50% of the subjects from each tank were fitted with VI tags (Northwest Marine Technology Ltd.) implanted in the post-ocular eyelid tissue (Niva, 1995). Following a further two weeks of acclimation, the fish were exposed to a range

of feeding regimes using commercial feed (EWOS Dynamic Red, size L) for a period of 27 weeks (beginning in January 2000). The feeding regimes were derived from a value of 0.5% of initial body weight per day (bwpd), which is comparable to commercial rations. Three tanks were fed 0.5%bwpd, two tanks were fed on a maintenance ration of 0.15%bwpd, and three tanks were fed on levels between maintenance and commercial rations; 0.25%bwpd, 0.35%bwpd and 0.45%bwpd. The remaining two tanks were fed to excess on 0.6%bwpd. For the purpose of analysis, the 0.15%bwpd and 0.25%bwpd feeding regimes were termed 'low' and the 0.45%bwpd, 0.5%bwpd and 0.6%bwpd feeding regimes were termed 'high'.

6. 2. 2. 1 Selection of subjects for part two

During part 1 the subjects were sampled 3 times; after 13, 21 and 29 weeks. Individuals were anaesthetised (Aqui-S – New Zealand), weighed, measured and identified. Data collected at samplings during weeks 13 and 21, together with information from growth tables (EWOS), enabled weight ranges to be defined for three new populations. Figure 6.1 summarises the movement of fish between parts 1 and 2. The populations of similar sized fish that were created after 29 weeks combined the best growing fish (good performers) from low feeding regimes, with the poorest growing fish (poor performers) from high feeding regimes. After 29 weeks, fish within the predetermined weight ranges were selected during sampling and VI tags were fitted to those that had not been tagged in part 1. The subjects were selected from seven tanks; one tank fed 0.6%bwpd, three tanks fed 0.5%bwpd, one tank fed 0.25%bwpd and two tanks fed 0.15%bwpd. The subjects from the three remaining undisturbed tanks were used to monitor feeding behaviour (chapter 3). All selected fish had grown during the initial experiment (unlike those described in chapter 4), and there was no evidence of injury. However, four subjects were removed from the experiment at the end of phase I as they had developed severe skin injuries. These individuals were also removed from all analyses. In addition, as eye injuries have been shown to affect growth in part 1 (see chapters 2 and 5), 14 fish sustaining eye damage were removed

from all analyses. Skin damage was not found to affect growth so fish with minor skin damage were not removed from the data set.

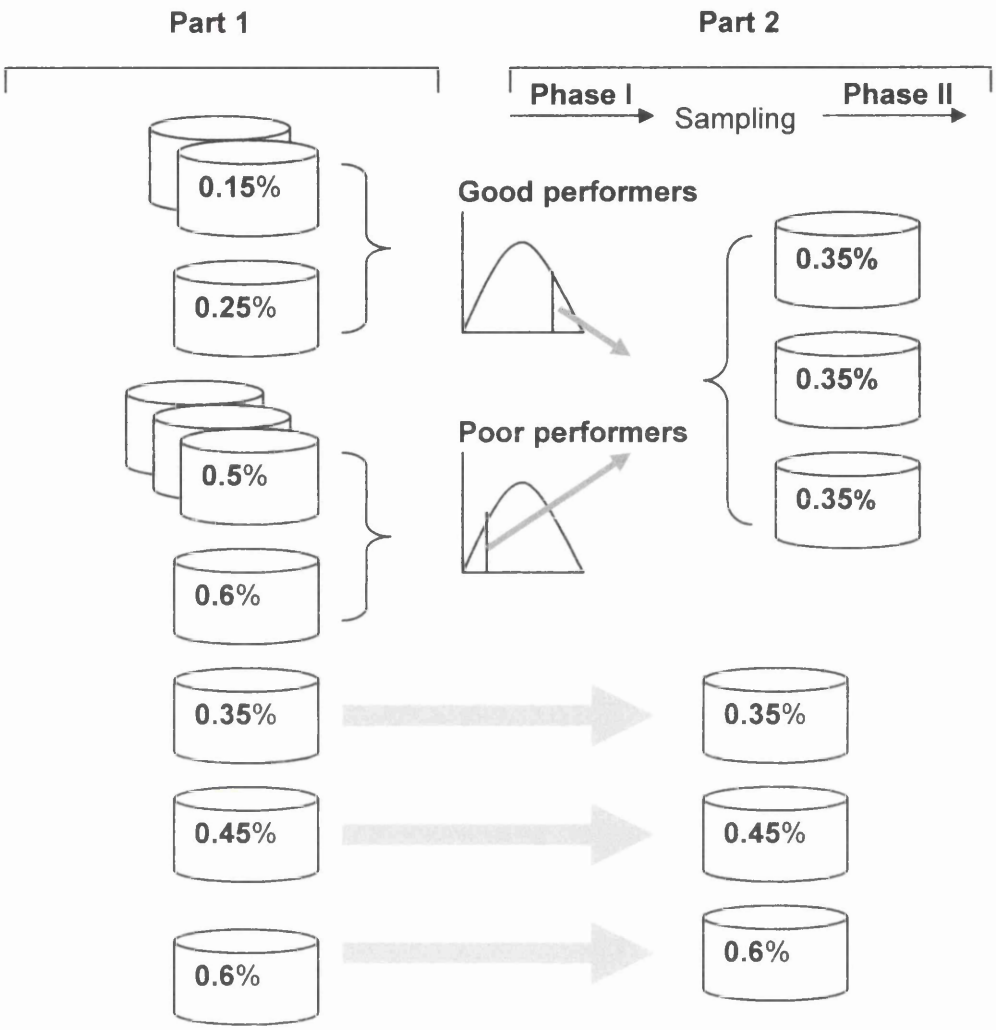


Figure 6.1 Summary of methods.

In order to minimise the size variation in each tank, the weight range used to select fish differed. The tanks containing the smallest, intermediate and largest fish were termed Sm, Lg and Med respectively to reflect the size of the fish at the start of part 2 without precluding their eventual size (median and interquartile ranges: tank Sm = 2.05,0.22; tank Med = 2.37,0.27; tank Lg = 2.68,0.21).

6. 2. 3 Part 2. Performance of size matched fish

6. 2. 3. 1 Temperature and daylength

The temperature range and the number of hours between dawn and dusk during part two are presented in Figure 6.2. As the water was pumped from a depth of 30m to decrease temperature variation, the median temperature was lower during phase I (median = 8.1°C, range = 7.5 - 11.5°C) than phase II (median = 13.6 °C, range = 9.1 - 14.1°C). In contrast, the number of hours between dawn and dusk was greater during phase I (median = 16.45 hours, range = 14.25 – 18.32) than phase II (median = 11.30 hours, range = 8.42 – 14.20).

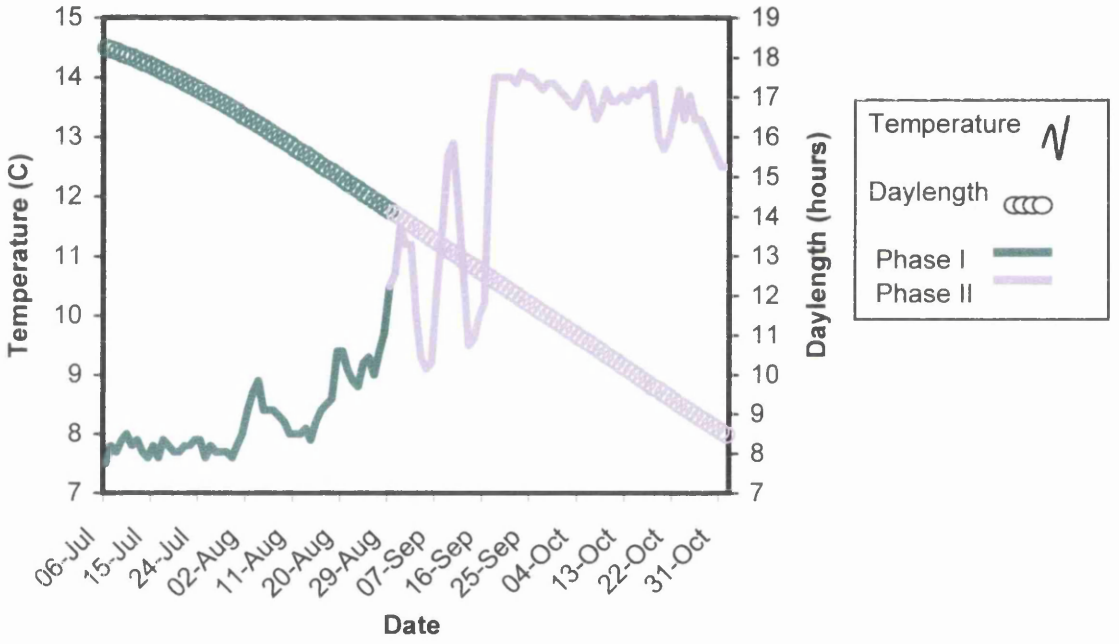


Figure 6.2 Temperature and daylength (expressed as the number of hours between dawn and dusk) for each time period in Part 2.

6. 2. 3. 2 Feeding regime and growth comparison group

The selected fish were housed in three tanks and fed at an intermediate feed level of 0.35%bwpd for 17 weeks. This level reversed the competitive conditions of both good and poor performing fish; competition decreased for good performing fish and increased for poor performing fish. It was chosen as it was unfamiliar to fish from high and low feeding regimes and promoted competition for food. In addition, the subjects in one of the three tanks used to monitor feeding behaviour (chapter 3) during part 2

were used, as a form of control, to compare growth rates of poor and good performers in part 2. The tank used for comparison was fed on 0.35%bwpd during part 1 and part 2; the same ration as the size-selected fish in part 2. At the start of part 2 the weight of the subjects in this tank did not differ in from the weight of the selected good and poor performers (Mann-Whitney U test: U = 2640, Z = -1.247, P = 0.212). Sampling was carried out for all tanks during weeks 8 and 17; the end of phase I and phase II respectively. During sampling all fish were anaesthetised, identified, weighed and measured.

6. 2. 4 Condition and growth

Growth in weight was calculated using Thermal Growth Coefficient according to the following formula (Cho, 1992):

$$TGC = ((W_{t1}^{1/3} - W_{t0}^{1/3}) / T \times D)1000$$

Where W_{t0} represents weight in grams at time 0 and W_{t1} weight in grams at time 1, T, mean temperature in °C, and D, number of growth days.

Growth in length was calculated using the same equation as growth in weight (see above), but substituting start and end length values instead of start and end weight values.

A relative condition score of each fish was calculated by plotting a regression line of weight and length. The regression line was created using fish with a similar size range to the good and poor performers selected for part 2. In addition, only fish that were grown on feeding regimes similar to commercially feeding regimes were used. To do this, data from the last sampling in part 1, from fish exposed to commercial feeding regimes, along with data from two of the populations used to monitor feeding behaviour in part 2 was used. Lower and upper mode fish were removed from the data set as

they affected the slope of the regression line. The regression line of ln (weight) on ln (length) was plotted ($F_{1,467}$ = 2206.03, R^2 = 0.83, P < 0.001) and the condition for each fish was defined as the residual weight, for a given length, from this regression line (i.e. the difference between the observed and the expected weight). Thus, fish with heavier weights than expected had a positive condition, while those with lower weights than expected had a negative condition. Change in condition was calculated by subtracting condition at one sampling from condition at the next sampling.

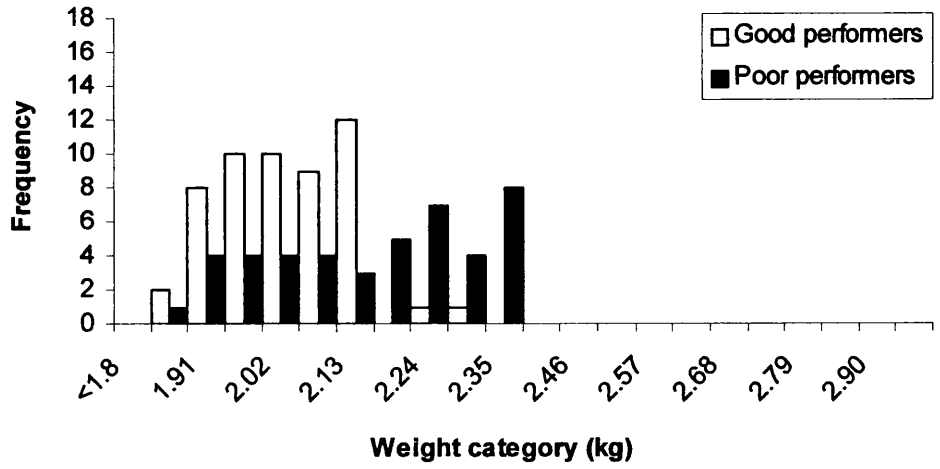
6. 2. 5 Data analysis

6. 2. 5. 1 Implications of analysis of phase I

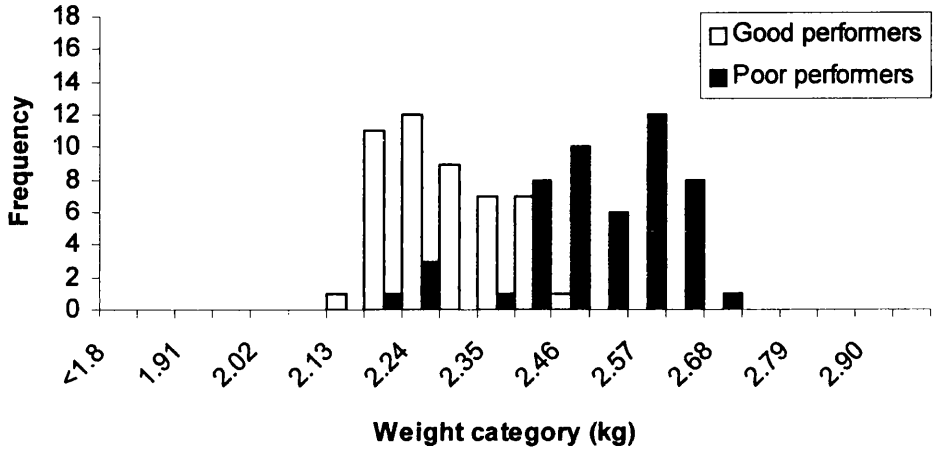
A two way ANOVA with tag (presence or absence) and previous regime (low or high) as factors revealed that there was no effect of previous tagging on the start weight of good performers or poor performers in any of the tanks ($F_{1,200}$ = 0.713, P = 0.399), nor was there an interaction effect between tagging and previous regime on weight ($F_{5,200}$ = 2.131, P = 0.203).

To test for a statistical difference in the initial weights of the population of each tank and also for an initial difference between fish with different growth history, a two-way analysis of variance (ANOVA) was performed with tank (Sm, Med or Lg) and previous performance (good or poor) as factors. This revealed a statistical difference, not only in the weight of fish in the three tanks ($F_{1,291}$ = 108.320, P < 0.001), but also a difference in the weight of good performers and poor performers ($F_{1,291}$ = 118629.9, P < 0.001) and an interaction between the effects of tank and previous regime ($F_{1,291}$ = 620.924, P < 0.003). In each tank, the good performers had lower weights than the poor performers and the difference between good and poor performers was greatest in tank Lg and smallest in tank Sm (Figure 6.3).

a) Sm tank



b) Med tank



c) Lg tank

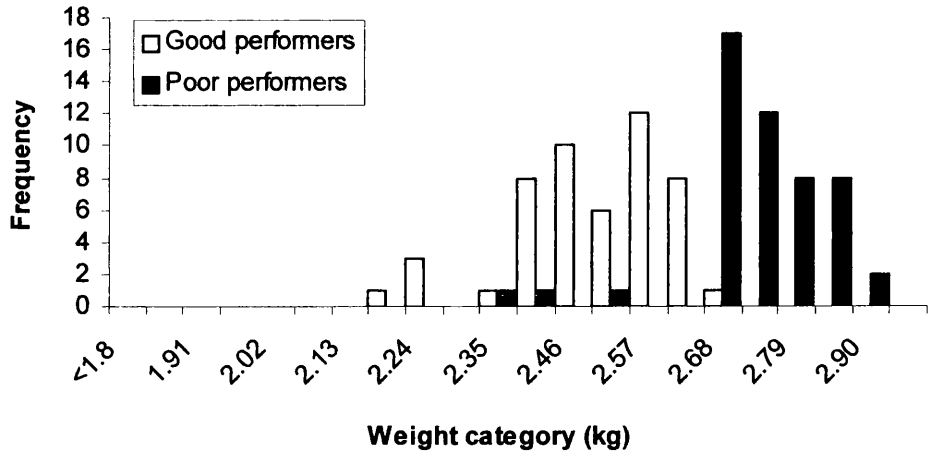


Figure 6.3. Initial weight frequency distribution for the selected populations of previously good and previously poor performing fish for a) tank Sm, b) Tank Med and tank Lg.

As smaller fish tend to grow faster than larger fish (Jobling, 1985), data sets were created for each tank in which there were no statistical differences in the weight of good performers and poor performers. This allowed comparison of growth without the influence of starting weight. The weight-adjusted data sets were created by removing the smallest good performers and the largest poor performers from the analysis until a t-test revealed that there was no longer a difference in the starting weight of good performers and poor performers in each tank (Figure 6.4a; tank Sm: $t_{57} = -0.740$, $P = 0.462$; tank Med: $t_{15} = -1.786$, $P = 0.094$; tank Lg: $t_{56} = -1.946$, $P = 0.057$). This practice resulted in a decrease in the number of data points in each tank. As a result, the number of individuals used in the analysis were; tank Sm: 36 good performers, 23 poor performers; tank Med: 9 good performers, 8 poor performers; tank Lg: 21 good performers, 37 poor performers.

Within the adjusted data sets there were differences in length and condition between good performers and poor performers in tanks Sm and Lg, but not in tank Med. In tanks Sm and Lg, good performers were longer than poor performers (Figure 6.4c; tank Sm: $t_{57} = 2.574$, $P = 0.013$; tank Med: $t_{15} = 0.810$, $P = 0.430$; tank Lg: $t_{56} = 2.829$, $P = 0.006$). As a consequence, good performers had a lower condition than poor performers in tanks Sm and Lg, but there was no difference in tank Med (Figure 6.4b; tank Sm: $t_{57} = -3.182$, $P = 0.002$; tank Med: $t_{15} = -1.204$, $P = 0.247$; tank Lg: $t_{56} = -3.900$, $P < 0.001$). The weights in the adjusted data set were then compared to the weights of comparison fish to ensure that comparison between the two data sets was still feasible. This revealed no difference between the adjusted data set and the comparison fish at the start of the study (Mann-Whitney U test: $U = 5919.00$, $Z = -1.235$, $P = 0.217$).

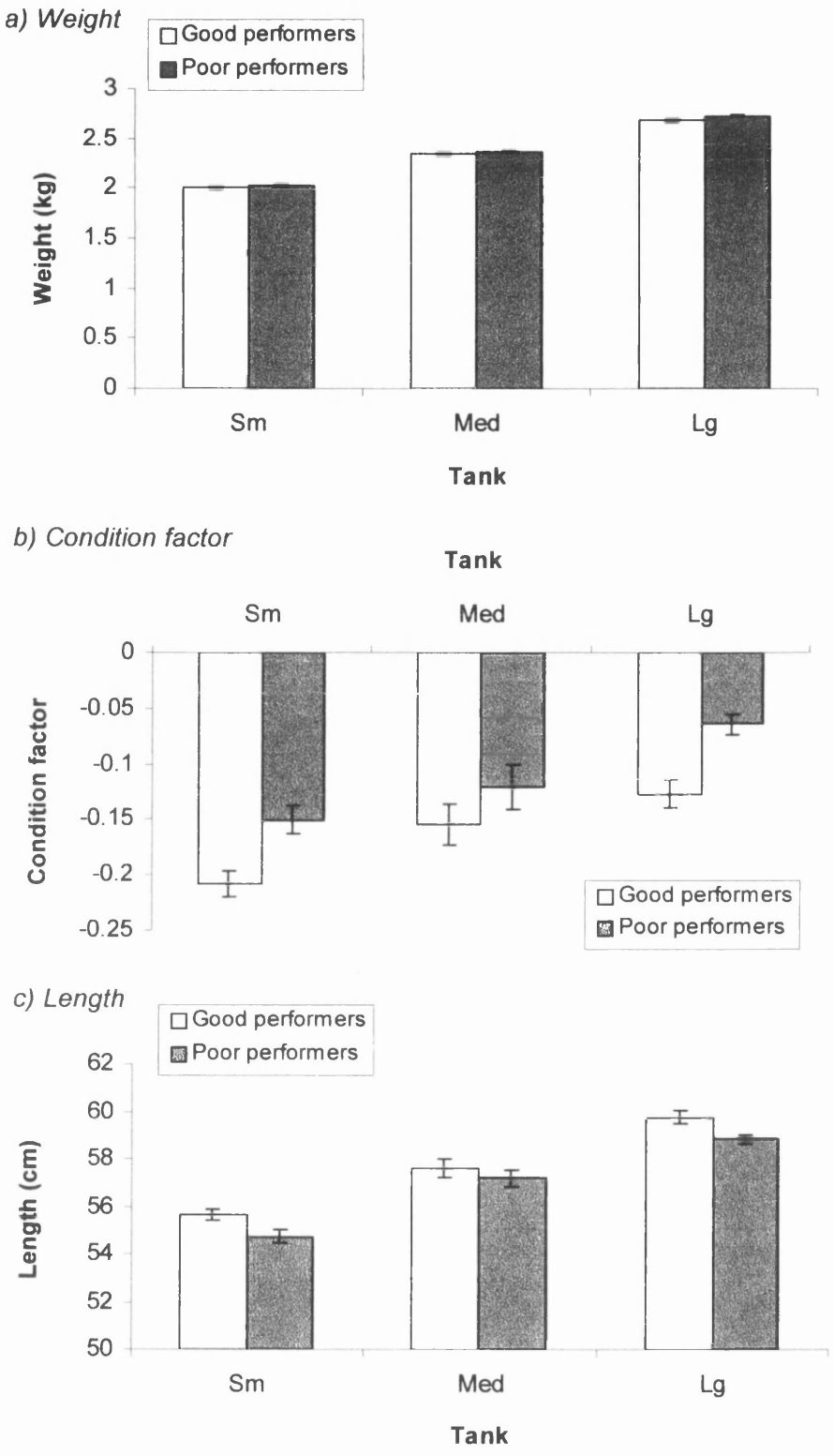


Figure 6.4 Initial differences in a) weight, b) condition and c) length of good and poor performers in tank Sm, tank Med and tank Lg

Similarly, at the start of phase II of part 2, independent samples t-tests were carried out for each tank to test for differences in weight of good performers and poor performers.

These tests found no difference in the weight of good performers and poor performers in tanks Sm and Med (Sm tank: t_{57} = 0.122, P = 0.903; tank Med: t_{15} = 1.035, P = 0.317). However, in tank Lg, good performers were heavier than poor performers (mean ± SE: good performers = 3.52 ± 0.093, poor performers = 3.20 ± 0.052; t_{56} = 3.236, P = 0.002). Therefore, fish were removed from the tank Lg data set until there was no difference between good performers and poor performers. After the removal of 4 subjects from the data set there was no longer a significant weight difference between good performers and poor performers ($t_{23.6}$ = 1.521, P = 0.185).

6. 2. 5. 2 Comparison of growth rate between good and poor performers

All results presented are from size corrected data from Part 2. All means are quoted with standard error of the mean (SEM) and all medians are quoted with interquartile ranges. As the size-range differed in the three experimental tanks, it was not possible to separate the effects of tank and size in each tank. Therefore, the effects of previous performance and tank/size on growth (in terms of weight, condition and length) were investigated using a two-way ANOVA for each tank. A one-way ANOVA was used to test for differences in growth between the established population and the size-matched populations, fed on the same ration level. At the end of phase I and phase II, a series of independent samples t-tests were used to test for differences in size (in terms of weight, length and condition) between good and poor performers in each tank.

6. 2. 5. 3 Relationship between initial nutritional status and growth

In order to investigate the relationship between initial nutritional status (expressed as condition and size) and growth, the initial weight, length and condition score were plotted against the change in each of these variables. This analysis was carried out separately for each tank for good performers and poor performers in each tank. As this resulted in a large number of regressions (18) a Bonferroni sequential correction (Sokal & Rholf, 2001) was applied to the data. When similar trends were found between groups and tanks, data were combined.

6. 3 RESULTS

6. 3. 1 Comparison of growth rate between good and poor performers

6. 3. 1. 1 Phase I

Analysis of growth in weight at the end of phase I revealed that good performers had a significantly higher weight increase than poor performers (Figure 6.5a; two way ANOVA, $F_{1,128} = 9.174$, $P = 0.003$). In addition, there was an effect of tank/size; the highest growth rates were found in tank Med and the lowest in tank Lg ($F_{2,128} = 10.897$, $P < 0.001$). Furthermore, there was an interaction between the effects of tank and previous performance history; the difference in weight increase of good and poor performers was largest in tank Lg and smallest in tank Sm ($F_{2,128} = 3.391$, $P = 0.037$).

Good performers also showed a significantly higher increase in condition than poor performers (Figure 6.5b; $F_{1,128} = 17.721$, $P < 0.001$). In addition, there was also a significant tank/size effect; tank Lg had the lowest increase in condition and tank Med the highest ($F_{2,128} = 19.818$, $P < 0.001$). However, there was no interaction between the effects of tank and previous performance history ($F_{2,128} = 0.948$, $P = 0.390$).

In contrast to the observed change in weight and condition, there was no difference in the length increase of good performers and poor performers ($F_{1,128} = 0.221$, $P = 0.639$). There was however, an effect of tank/size; tank Med had the greatest increase in length and tank Lg the least (Figure 6.5c; $F_{2,128} = 1.427$, $P = 0.031$). Furthermore, there was an interaction between the effect of tank and previous performance history as good performers increased in length to a greater extent than poor performers in tanks Med and Lg. In tank Sm the opposite occurred: good performers increased in length to a lesser extent than poor performers ($F_{2,128} = 3.735$, $P = 0.027$).

A one-way ANOVA with *post hoc* LSD test was used to compare the growth rates of good and poor performers in each tank with an established (comparison) population fed on the same feeding regime. The test revealed that there was a difference in the growth rates of the good and poor performers from each tank and the comparison fish ($F_{6,167}$ = 7.630, P < 0.001). However, the *post hoc* LSD test revealed that this difference only represented a significantly lower growth rate in the poor performers in tank Lg than the comparison population (Figure 6.5a).

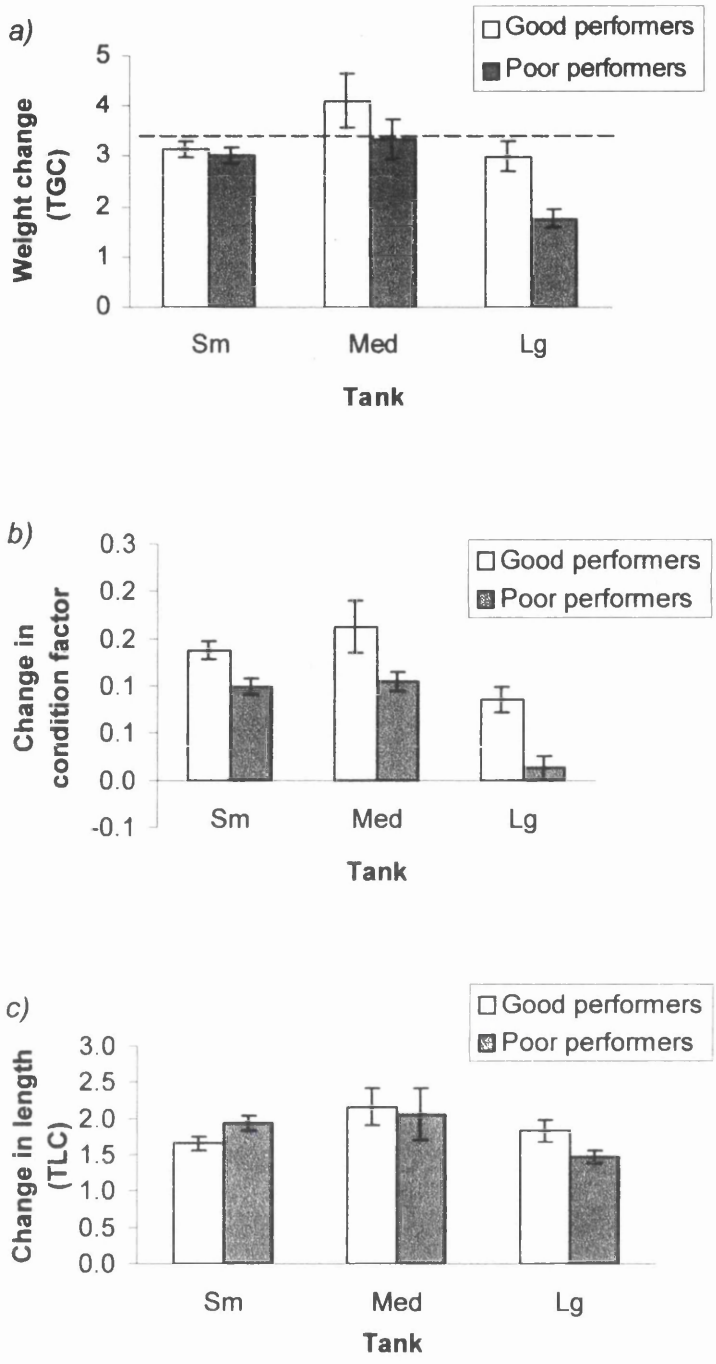
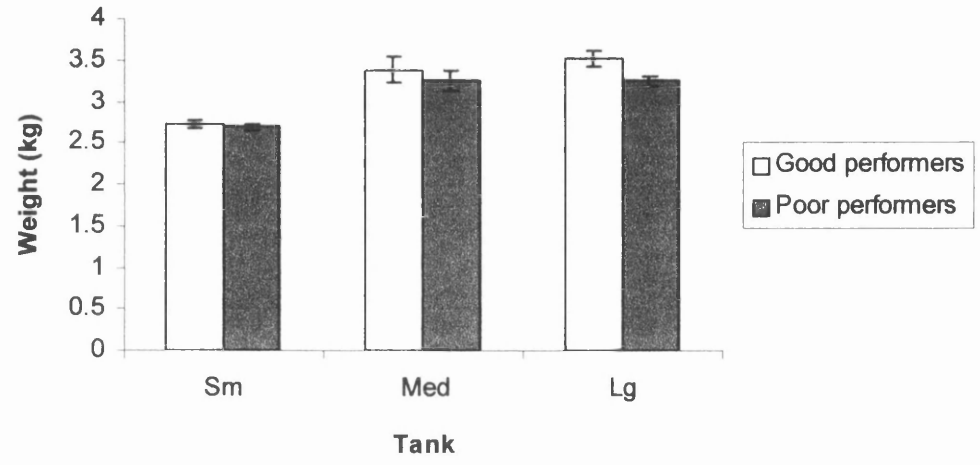


Figure 6.5 Change in a) weight, (dashed line indicates growth of comparison group of fish) b) condition and c) length of good and poor performers in tank Sm, tank Med and tank Lg during phase I.

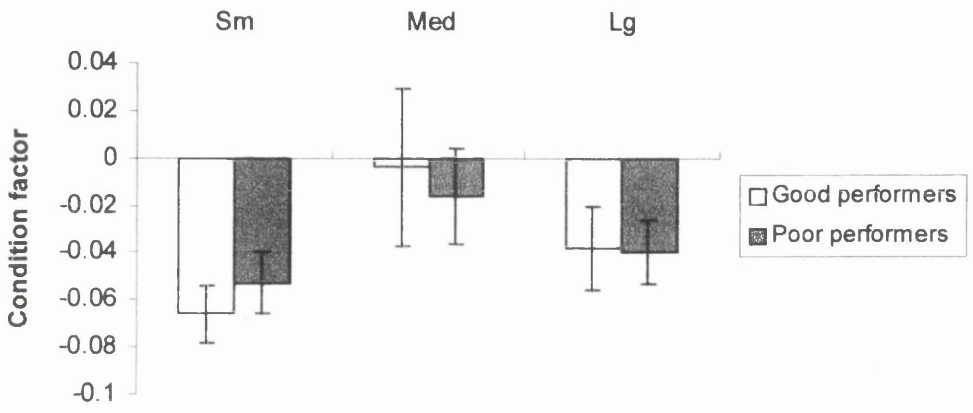
At the end of phase I, two-way ANOVA tests were used test the effects of previous performance and current tank / size on weight, length and condition of the subjects. In terms of weight, there was a significant effect of tank and performance, and a significant interaction between tank and performance (Figure 6.6a; Table 6.1a).

Overall, good performers were heavier than poor performers. In addition, fish in the Lg tank were the largest and those in the Sm tank were the smallest. The interaction term indicated that the weight difference between good and poor performers was different across the tanks; the greatest weight difference was observed in tank Lg and the smallest in tank Sm (Figure 6.6a; Table 6.1a). The analysis carried out on condition revealed that there was an effect of tank / size, but there was no difference in the condition of good and poor performers, nor was there an interaction between tank and regime (Figure 6.6b; Table 6.1b). Subjects in the Med tank was the lowest condition and those in the Lg tank had the highest condition. Lastly, in terms of weight there was a significant effect of tank and of previous performance, but there was no interaction effect (Figure 6.6c; Table 6.1c). Overall, good performers were longer than poor performers, and the fish in the Lg tank were the largest and the fish in the Sm tank were the smallest (Figure 6.6c; Table 6.1b).

a) Weight



b) Condition factor



c) Length

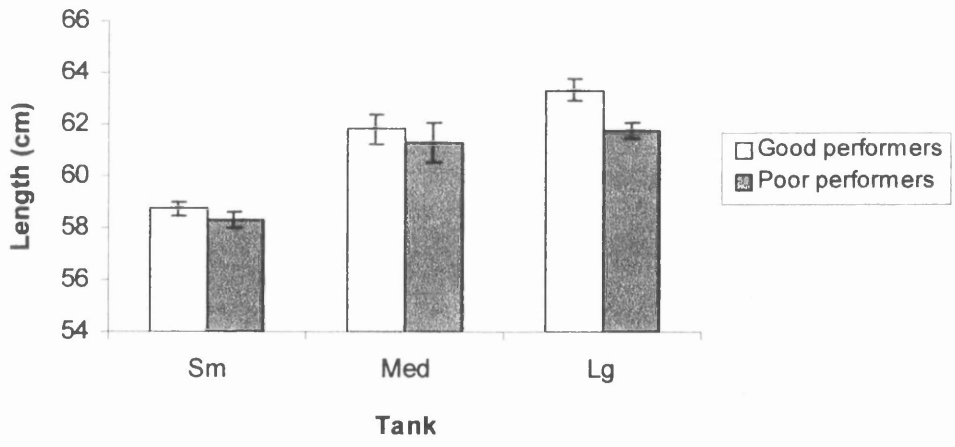


Figure 6.6 a) weight, b) condition and c) length of good and poor performers in tanks Sm, Med and Lg at the end of phase I.

Table 6.1 Summary of results from two-way ANOVA tests on the effect of performance and tank / size on a) weight, b) condition and c) length

a) Weight

	F	d.f.	*P*
Performance	7.257	1,128	*0.008*
Tank / size	68.788	2,128	*< 0.001*
Performance*Tank	3.367	2,128	*0.038*

b) Condition

	F	d.f.	*P*
Performance	7.257	1,128	*0.008*
Tank / size	68.788	2,128	*< 0.001*
Performance*Tank	3.367	2,128	*0.038*

c) Length

	F	d.f.	*P*
Performance	0.111	1,128	*0.739*
Tank / size	3.685	2,128	*0.028*
Performance*Tank	0.775	2,128	*0.463*

6. 3. 1. 2 Phase II

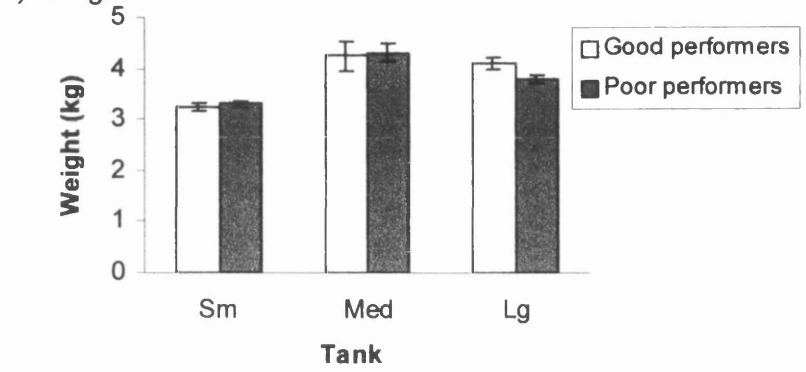
The growth rates observed during phase II were markedly lower than those observed in phase I (Mean ± SE for all fish: phase I = 2.85 ± 0.12, 17 weeks = 1.05 ± 0.06; paired samples t-test: $t_{122} = 15.140$, $P < 0.001$). During phase II there was no difference in the increase in size, in terms of increase in weight, length or condition, between good and poor performers (Table 6.2). In contrast, a difference was observed between tanks for each measure of size increase; the greatest increase in length and weight was observed in tank Med and the lowest increase in weight and length was observed in tank Lg (Table 6.2). The smallest decrease in condition was observed in tank Sm and the largest decrease in condition in tank Med. There were no interaction effects between tank/size and previous performance in any tank (Table 6.2)

At the end of phase II, there were no differences in the weight, length or condition of good and poor performers (Figure 6.7; Table 6.3). There was an effect of tank / size on weight, length and condition; tank Med had the greatest weight, length and condition (Figure 6.7; Table 6.3). In addition, there was a marginally significant interaction between tank and previous performance on weight and length, but not condition; the greatest difference between good and poor performers was in tank Lg (Figure 6.7; Table 6.3).

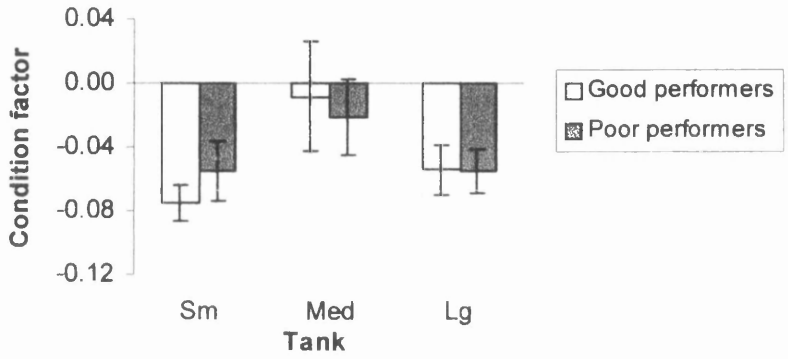
Table 6.1 Summary of results from two-way ANOVA on effects of previous performance (good or poor), tank and the interaction of these effects on growth in weight (TGC), growth in length (TLC) and change in condition during phase II.

			Effect tested		
Growth variable			Performance	Tank	Interaction
TGC	ANOVA results		$F_{1,117} = 1.142$, $P = 0.287$	$F_{2,117} = 11.696$, $P < 0.001$	$F_{2,117} = 1.067$, $P = 0.347$
	Mean ± SE	Tank Sm		1.06 ± 0.07	
		Tank Med		1.58 ± 0.15	
		Tank Lg		0.852 ± 0.054	
TLC	ANOVA results		$F_{1,117} = 1.559$, $P = 0.214$	$F_{2,117} = 11.323$, $P < 0.001$	$F_{1,117} = 1.383$, $P = 0.255$
	Mean ± SE	Tank Sm		0.992 ± 0.040	
		Tank Med		1.221 ± 0.095	
		Tank Lg		0.843 ± 0.028	
Ch CF	ANOVA results		$F_{1,117} = 1.559$, $P = 0.214$	$F_{2,117} = 11.323$, $P < 0.001$	$F_{1,117} = 1.383$, $P = 0.255$
	Mean ± SE	Tank Sm		-0.005 ± 0.008	
		Tank Med		-0.028 ± 0.012	
		Tank Lg		-0.019 ± 0.075	

a) Weight



b) Condition factor



c) Length

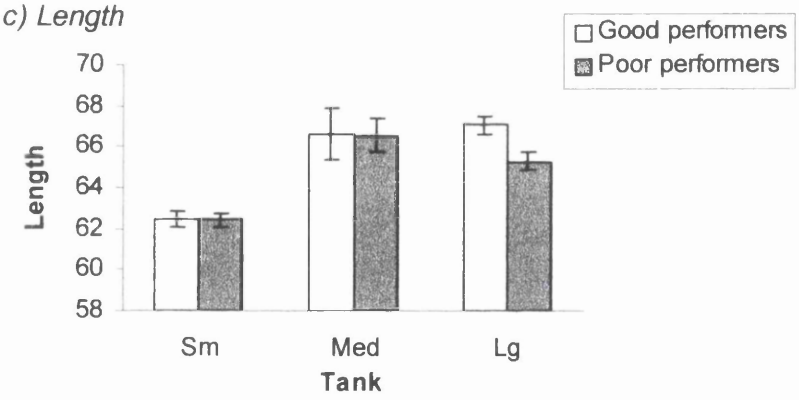


Figure 6.7 a) weight, b) length and c) length of good and poor performers in tank Sm, tank Med, tank Lg at the end of phase II.

Table 6.3 Summary of results from two way ANOVA tests on the effect of performance and tank / size on a) weight, b) condition and c) length

a) Weight

	F	d.f.	*P*
Performance	0.732	1,117	*0.394*
Tank / size	39.527	2,117	*< 0.001*
Performance*Tank	3.174	2,117	*0.045*

b) Condition

	F	d.f.	*P*
Performance	1.088	1,117	*0.299*
Tank / size	5.670	2,117	*0.004*
Performance*Tank	0.454	2,117	*0.636*

c) Length

	F	d.f.	*P*
Performance	2.479	1,117	*0.118*
Tank / size	75078.686	2,117	*0.001*
Performance*Tank	2.479	2,117	*0.050*

6. 3. 2 Relationship between nutritional status and growth

6. 3. 2. 1 Phase I

The effect of nutritional status, expressed as fish size and condition, on the change in each of these variables was investigated. However, the use of a sequential Bonferroni sequential correction (Sokal & Rohlf, 2000) revealed that there were no significant relationships between nutritional status and growth; there was no relationship between weight, condition and length and the increase in each of these variables. However, the regressions that were plotted indicated that there was a trend for individuals with a lower initial condition to have a greater increase in condition. This trend was observed

in good and poor performers in each tank, and indeed, good and poor performers for all tanks were combined there was a significant negative relationship between initial condition and change in condition ($F_{1,32}$ = 58.874, R^2 = 0.308, P < 0.001). The results from the series of regressions performed are presented in Table 6.4.

Table 6.4 Summaries of results of regression analysis of the relationship between initial nutritional status, (a) weight, b) condition, c) length) and the increase of each variable during phase I. Regressions were carried out separately for previously good and previously poor performers from each of the three experimental tanks.

a) Weight

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	4.507	1,34	0.117	*0.041*	0.568	1,21	0.026	*0.459*
Med tank	2.369	1,7	0.253	*0.168*	0.011	1,6	0.002	*0.921*
Lg tank	4.000	1,19	0.174	*0.060*	0.302	1,35	0.009	*0.586*

b) Condition

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	8.835	1,34	0.206	*0.005*	3.313	1,21	0.136	*0.083*
Med tank	0.090	1,7	0.013	*0.771*	0.482	1,6	0.074	*0.514*
Lg tank	0.647	1,19	0.033	*0.431*	2.303	1,35	0.035	*0.138*

c) Length

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	2.067	1,34	0.057	*0.160*	0.964	1,21	0.044	*0.337*
Med tank	0.744	1,7	0.096	*0.417*	0.170	1,6	0.027	*0.695*
Lg tank	0.769	1,19	0.039	*0.391*	0.385	1,35	0.011	*0.539*

6. 3. 2. 2 Phase II

Similarly, there was no relationship between nutritional status, in terms of weight or length in phase II. However, the application of a Bonferroni sequential correction (Sokal & Rohlf, 2000), revealed that the poor performers in the Sm tank with the lowest condition showed the greatest increases in condition. The results from the series of regressions are presented in Table 6.5

Table 6.5 Summaries of results of regression analysis of the relationship between initial nutritional status (a) weight, b) condition, c) length) the increase in each variable during Phase II. Regressions were carried out separately for previously good and previously poor performers from each of the three experimental tanks.

a) Weight

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	1.480	1,31	0.046	*0.233*	0.791	1,21	0.036	*0.384*
Med tank	Sample size too small (n = 5)				0.622	1,6	0.094	*0.460*
Lg tank	2.926	1,19	0.133	*0.103*	1.384	1,29	0.046	*0.249*

b) Condition

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	11.609	1,31	0.272	*0.002*	0.964	1,21	0.044	*0.337*
Med tank	Sample size too small (n = 5)				0.170	1,6	0.027	*0.695*
Lg tank	6.532	1,19	0.256	*0.019*	0.385	1,35	0.011	*0.539*

c) Length

	Good performers				Poor performers			
	F	d.f.	R^2	*P*	F	d.f.	R^2	*P*
Sm tank	0.573	1,31	0.018	*0.455*	1.742	1,21	0.077	*0.201*
Med tank	Sample size too small (n = 5)				0.904	1,6	0.002	*0.904*
Lg tank	0.140	1,19	0.007	*0.712*	9.773	1,29	0.252	*0.004*

6. 4 DISCUSSION

6. 4. 1 Overview of results

The described study investigated the relationship between prior performance, and nutritional status and increase in size. The study was split into two phases, which were distinct in temperature, daylength and growth. The average growth rates of good and poor performers in phase II were 50% lower than in phase I. This growth difference in likely to have been caused by environmental influences.

Although the fish were held in tanks with a constant photoperiod, they may have received environmental light cues through the windows. During the study water temperature within the tanks increased and natural daylength decreased. Seasonal differences in feed intake and growth have been recorded in adult Atlantic salmon in commercial conditions (Forsberg, 1995; Blyth et al., 1999; Boeuf & Le Bail, 1999) under natural photoperiod. In addition, salmonids have been observed to display natural rhythms of growth when exposed to 12h light: 12h dark photoperiod regimes (Eriksson, 1978; Sæther *et al.*, 1996).

In the present study, it is likely that growth in period two was heavily influenced by environmental cues and that this effect may have masked differences in the growth of good and poor performing fish. In addition, the greatest effects of previous performance are likely to occur when the fish are initially placed in tanks containing individual of differing previous performance. By the end of phase I the fish had presumably restored any poor nutritional condition that they had when they started, and had gone through the most rapid phase of any compensatory response (see Broekhuizen, 1994). Therefore, only the results from phase I of part II are discussed here.

6. 4. 2 Comparison of growth rate between good and poor performers

Initially, (in phase I) fish that were previously good performers showed a greater increase in size, in terms of weight and condition, than individuals that were previously poor performers. The extent of the difference was greatest in the largest group of fish (tank Lg). This is compatible with the suggestion that the differences in growth in part 1 may be due to differences in competitive ability that also manifest themselves when good and poor performers are kept in size-matched groups.

The observed differences in competitive ability may have been confounded by past competitive experience. Studies on a wide range of animals have demonstrated that individuals that win in one contest are more likely to win in the following contest (coho salmon, Rhodes & Quinn, 1998; chickens Cloutier & Newberry, 2000; crab spiders, Dodson & Schwaab, 2001). Therefore, the increased growth of the good performers in the present study could be attributed to prior winning experience. In the current study, it is not possible to distinguish between the effects of prior competitive experience and intrinsic competitive ability. However, it is clear that prior competitive ability was a good predictor of performance in size-matched groups.

Both poor and good performers may have experienced growth suppression in Part 1; restricted food rations could restrict the growth of the good performers, and the growth of poor performers may have been suppressed by poor competitive ability or restricted feed intake imposed by better competitors. The growth rates of the size-selected fish in phase I were no different from those in the undisturbed population, indicating that they were not undergoing compensatory growth. However, the smallest fish of the good performers showed the greatest increase in both condition and weight, indicating that they may have been restricted by food levels previously, but could now (in part 2) exert their superior competitive status and catch up lost growth opportunities.

Consistent differences were found in the extent of size-increase in each tank; tank Med had the greatest increase in all size parameters measured and tank Lg had the lowest increase in all size parameters measured. In order to create size-matched populations, the size range in each tank differed. The good performers in tank Lg were the largest individuals from poor feeding regimes and were therefore better performers than those in tanks Med and Sm. Furthermore, the poor performers in tank Sm were the smallest individuals from high feeding regimes and therefore poorer competitors than those in tanks Med and Lg. Therefore, the consistent differences between tanks may have been due to the fact that the degree of overlap in performance was not uniform between tanks.

The subjects in the Lg tank had the lowest size-corrected growth rates of all the groups. This may have been due to increased competition; in small groups of juvenile Atlantic salmon, growth rates were reduced when a competitor was present (Huntingford *et al.*, 1993). The poor performers in tank Lg were the only group to have lower growth rates than the undisturbed, comparison population. The growth of these individuals may have been suppressed by the good performers. Additionally, the fact that the Lg tank had the smallest overlap in weight of good and poor performers may have influenced the differences observed between good and poor performers in this tank. In a study investigating metabolic rate as a predictor of competitive ability in pairs of juvenile rainbow trout, McCarthy (2001) found that the share of the meal that each competitor obtained approached equality when the competitors were more evenly matched (in terms of metabolic rate). In the described study, the Lg tank is the least well matched with respect to size and therefore competitive ability. This may have resulted in the increased differences observed in this tank.

Overall, there were no differences in the length-increase of good and poor performers. However, in contrast to the other populations, good performers in the population containing the smallest individuals had a lower increase in length than poor performers.

The good performers in this tank had the lowest condition of all groups in the study. Jobling and Koskela (1996) found that the smallest individuals (in Arctic charr) exhibited the greatest recovery after a period of food restriction and Nicieza *et al.*, (1993 & 1994) found that the smallest Atlantic salmon grew fastest prior to smolting. In addition, MacLean and Metcalfe (2001) suggested that individuals had a target body size for a given time of year. If this were also true for condition, the group of good competitors with the lowest condition would be furthest away from their target and most likely to show a recovery response. The good performers in question do increase in weight condition more than the poor performers in the same tank, but they do not show a markedly higher increase than good performers in the other tanks.

These results suggest that persistent individual differences in performance in adult Atlantic salmon can be used to predict subsequent growth. The differences in growth are short-lived, but they provide an increase in size. In groups of fish, a large body size can confer a competitive advantage (e.g. cichlids, Francis, 1988). Therefore, when an individual is introduced to new conditions, it is possible that the persistent ability of an individual to grow well would allow an initial growth spurt, leading to a long term competitive advantage.

6. 4. 3 Relationship between nutritional status and growth

In phase I, similar trends in growth with respect to nutritional status were observed in all tanks in terms of condition. However, there was no relationship between initial length and increase in length in good or poor performers in any tank.

The trend observed for condition was similar in all tanks. Individuals with lower condition tended to show a greater increase in condition. All individuals selected for the study had previously undergone a period of growth suppression. In good performers this was due to restricted feed rations and in poor performers this was due to poor competitive ability or restricted ration imposed by better competitors. In the

present study, feed rations were higher than those previously experienced by good performers. In addition, poor performers had a size advantage at the start of the part 2. Therefore, it is likely that both groups would undergo some form of compensatory growth upon introduction to new, less competitive conditions. There was no evidence of increased growth rates in the present study, but the subjects may have undergone a compensatory increase in condition. During compensatory growth, individuals that have undergone the greatest degree of growth depensation are likely to undergo the greatest degree of compensatory growth (Jobling and Koskela, 1996). In the described study, individuals with the lowest condition at the start of part 2 showed the greatest increases in condition. Similarly, previous authors have demonstrated a negative relationship between fat reserves and appetite in juvenile Atlantic salmon (Miglavs & Jobling, 1989; Metcalfe & Thorpe, 1992; Bull *et al.*, 1996) and between fat reserves and weight gain (Metcalfe & Thorpe, 1992; Sæther & Jobling 1999).

In the tank with the largest fish, there were no statistically significant relationships between nutritional status and growth in terms of weight, length or condition. However, the (non-significant) trends observed in good performers from this tank were in the opposite direction to those observed in other tanks; fish that were heavier tended to increase more in condition and weight. This result is contrary to the general trend for smaller fish to grow more (Jobling, 1985), and suggests that effects other than body size are influencing the growth of this group of fish. In large groups, social hierarchies are less stable (Fenderson & Carpenter, 1971) and there may be a constantly changing sub-group of individuals competing at the top end of the hierarchy instead of one single individual (Brannas and Alanara, 1996; MacLean *et al.*, 2000). Therefore, as the good performers in tank Lg are the largest, these may represent this sub-group of dominant individuals.

In terms of length (for good and poor performers), shorter fish tended to increase more in tanks Sm and Med. In contrast, the poor performers in tank Lg that were the

smallest increased less. In part 1 of this study; the experiment that supplied the fish for the present experiment, a small group of fish did not increase in weight over the duration of the study (29 weeks). These fish were found to be increasing in length during this time. Resources may be preferentially allocated to skeletal mass in times of food restriction as mass is easier to increase in times of plentiful food supply. Nicieza and Metcalfe (1997) also found that juvenile Atlantic salmon increased in length and decreased in weight while subjected to food restriction. The subjects then restored the lost condition within a week when normal feeding levels were restored.

The observed persistent, size-independent differences in competitive ability can confer at least, short-term increases in growth rate. The resulting increase in body size may confer competitive advantages. Abbott *et al.* (1985) found that a size-advantage of 5% resulted in the largest of two juvenile steelhead trout becoming dominant. Large body-size is also related to dominance in the domestic goat (Barroso *et al.*, 2000) and provided a mating advantage for male medaka (Howard *et al.*, 1998). Nutritional status, in terms of condition may also influence individual growth as individuals with lower condition were more likely to increase in condition.

6. 4. 4 Conclusions and implications

In conclusion, persistent differences in competitive ability can confer a growth advantage, at least in the short term. Individual performance within a group will depend on the competitive ability of the other individuals within the group. Therefore, if it were possible to mass screen competitive ability or some other related trait, groups of individuals with particularly good performance could be identified and possibly removed earlier, encouraging the growth of other poorer competitors.

CHAPTER 7. GENERAL DISCUSSION

A number of issues, of both fundamental and applied scientific interest, were investigated during this study. Specifically, the nature and causes of variability in growth rates were considered in Atlantic salmon in large, high density groups. In terms of fundamental biology, these groups provided the opportunity to investigate resource acquisition and utilisation.

7. 1 Resource acquisition and utilisation

7. 1. 1 Dominance in large groups

In salmonids, and indeed in many other species, competition is structured in the form of social hierarchies (e.g. rabbits, Albonnetti *et al.*, 1988; Willow tits, Ekman & Askenmo, 1984; Atlantic salmon, Keenleyside and Yamamoto, 1962). However, one might expect that individual defence of resources would be difficult in large groups. In keeping with this, previous authors have demonstrated that hierarchies are less stable in large groups (Fenderson & Carpenter, 1971; MacLean *et al.*, 2000). However, this study demonstrated the occurrence of social hierarchies in large groups of Atlantic salmon.

In initially tightly graded populations, individuals that underwent the greatest increase in weight were found to have a lower brain stem serotonin turnover (indexed by the ratio of 5-HIAA/5-HT) than those that only increased in weight by a small amount (Chapter 4). Previous studies on small groups of salmonids revealed that dominant individuals had a lower serotonin turnover and subordinate individuals had a higher serotonin turnover (Winberg *et al.*, 1992a; Winberg & Nilsson, 1993). Therefore, the observed

results suggest the occurrence of a dominance hierarchy within these large groups at high densities.

Dominant individuals usually gain preferential access to resources both temporally (e.g. Eurasian badgers, Revilla & Palomares, 2001; brown trout, Alanärä *et al.*, 2001) and spatially (female red deer, Clutton-Brock *et al.*, 1984; mountain goats, Cote & Festa-Bianchet, 2001). In the described study, the largest individuals were observed to have a different vertical distribution from the smallest individuals and also to feed at different times from small fish (Chapter 3). This suggests that small fish were excluded from feeding at particular times of the day. In addition, as there was an increase in the proportion of large fish feeding and a decrease in the proportion of small fish feeding in low ration levels, it would appear that smaller fish were prevented from feeding when the populations were exposed to low ration levels. This is in agreement with previous studies showing that dominance hierarchies are more pronounced under reduced rations (Moutou *et al.*, 1998; Alanärä *et al.*, 2001).

During the development of size differences, it became apparent that there were distinct sub-groups of fish displaying different growth trajectories (Chapter 2). Those that were in the lower mode and thus showed very little increase in weight were analogous to subordinate fish in terms of behaviour and social history, whereas those that were in the upper mode were analogous to dominant fish in terms of behaviour and social history. Chapter 2 also revealed that individuals moved within the distribution over time, this indicated that the strategy or status of the individuals concerned was labile.

7. 1. 2 Intrinsic variation in competitive ability

Chapter 2 provided an initial indication of intrinsic variation in competitive ability, as initially tightly size-graded individuals showed a marked increase in weight variation over a short space of time. In addition, there was a strong relationship between initial weight and growth that suggested that particular individuals were predisposed to

perform well. This was confirmed in Chapter 6 as intrinsic competitive ability was shown to provide an initial growth spurt and therefore a weight advantage over individuals that were intrinsically poor performers. It is possible that prior winning experience, observed in a wide range of animals (e.g. coho salmon, Rhodes & Quinn, 1998; chickens, Cloutier & Newberry, 2000) could have influenced the observed intrinsic competitive ability. However, further studies are necessary to elucidate the exact mechanism behind the observed intrinsic competitive ability.

Although it was not possible to reject the occurrence of social suppression of growth by separating non-growing fish from larger fish (Chapter 5), evidence was presented that poor growing, subordinate individuals do have the ability to grow. Recently, there have been a number of studies investigating the relationship between metabolic rate and competitive ability and aggression. For example, McCarthy (2001) demonstrated that resting metabolic rate correlated with increased competitive ability in pairs of juvenile rainbow trout. In light of this, O'Connor et al (2000), observed that the standard metabolic rate of juvenile Atlantic salmon decreased during food deprivation and increased on when the food supply was increased. Although there was no difference in the food supply when previously non-growing fish were observed to rapidly increase in weight and condition, it is likely that they responded to seasonal cues and changed their growth trajectory, possibly as a result of an increase in metabolic rate.

7. 2 Welfare

Concerns about the welfare of farmed animals have grown in recent years. As a result of this, fish farming has begun to come under scrutiny, with the UK government funding projects investigating, amongst other things, humane slaughtering methods and the effect of high stocking densities on the welfare of farmed fish (FAWC, 1996). It is extremely difficult to study the behaviour and therefore the welfare of large groups of animals. Land animals can be tagged to allow visual observations e.g. earmarks on

groups of 20 domestic goats (Barrosso, 2000) and radio collars on 20 African elephants (Thouless, 1996). However, studying animals underwater presents greater problems in terms of reading and retrieving tags. Ultrasonic techniques have been developed and tested in sea cages, but involve the use of relatively large and expensive tags (Juell and Westerberg, 1993; Bégout-Anras, 1995). In addition, all of the above methods of studying social behaviour involve tracking animals until sufficient data is collected. In this study a technique, known to predict social dominance in small groups of salmonids (Winberg et al. 1992a; Winberg & Nilsson 1993) was applied to large groups of Atlantic salmon. This technique provides information on the past social history of individuals by measuring the ration of serotonin to its principle metabolite in the brain (5-HIAA/5-HT). Furthermore, as the neuroendocrine system is highly conserved across vertebrates, it is likely that the technique could be used to increase knowledge of social interactions in other large groups of animals e.g. domestic hens, wild deer or shoaling fish. For example, studies have shown that particular positions in the shoal are beneficial in terms of feed acquisition and energy efficiency (Krause, 1994). The status of individuals in these and in other positions could be ascertained using the described technique. The technique could also be used to investigate social interactions in other species of farmed animals, for example free-range hens.

The finding that there was a group of profoundly subordinate individuals that did not grow in large, high-density groups raises welfare concerns about the current methods of rearing salmonids for consumption and stocking. In aquaculture, and indeed in many intensive farming situations (e.g. broiler chickens), the rearing environment is unnaturally uniform. There are few, if any, places that subordinate individuals could escape harassment from dominant individuals. Höjesjö *et al.* (2002) demonstrated that, in groups of 10 brown trout fry, dominant individuals grew faster in simple habitats than in complex habitats, whereas subordinate individuals grew relatively better in complex habitats. This was presumably due to the fact that subordinate individuals can hide in complex habitats. The welfare of farmed salmon, and possibly other animals

reared in large groups, may benefit from the provision of some kind of shelter for smaller, poor performing individuals. However, further studies are necessary to assess the success of shelter provisioning in terms of growth improvement and economics.

7. 3 Implications for Atlantic salmon aquaculture

In terms of applied biology, this study addressed the problem of size variation in aquaculture. In particular, the size variation development was monitored and individuals that contributed to this variation were characterised.

As feed costs amount to 40% – 50% of total production costs in aquaculture (Sveier & Lied, 1998), information on the effects that increasing and decreasing feed levels have on fish is important. There was no difference, in terms of length, weight or condition, between populations that were underfed (90% of commercial level), overfed (120% of commercial level) or fed on commercial feed levels (Chapter 2). As the feed level was increased daily according to an estimate of growth (0.5% per day), the amount of food presented to the fish may not have actually been the predicted amount. Feeding patterns in salmonids change daily and seasonally, so a uniform increase in the amount of feed presented each day may have resulted in over feeding and underfeeding during points of the study.

In contrast, when populations were dramatically underfed (30 – 50% of commercial feed levels) the brain biochemistry of all individuals became more like that of subordinates (Chapter 4). Cages of fish may be underfed a result of feed quotas (imposed in Norway) and fish are usually starved before slaughter (see Einen *et al.*, 1998). During this time social interactions are likely to increase, resulting in increased levels of social stress in all fish. As stress increases disease susceptibility (Schreck 1981), extra vigilance (for example for outbreaks of sealice) should be applied when large groups of fish are starved or underfed.

Many individuals sustained injuries during the described studies. Although many of the injuries were a result of abrasion on tank fittings, injuries do occur in aquaculture conditions (Willoughby, 1999). This study revealed that the skin injuries did not affect growth of the fish, however eye injuries did. Furthermore, an individual with an eye injury at one sampling, was more likely (than an individual that did not have an eye injury at that sampling) to have an eye injury at the following sampling. These findings do not suggest that skin injuries do not affect fish at all, but rather that there were no obvious effects in growth over a minimum of 8 weeks.

In contrast, eye injuries were found to be detrimental to an individuals growth rate. This finding highlights the importance of removing all sources of abrasion in experimental tanks, especially on tank walls. Eye injuries are less likely to affect fish in cages as the rope that they are made of is not abrasive. However, care should be taken that cage furniture, such as waste feed monitors, biomass counters and cage cleaning appliances do not have abrasive parts. In addition, heavy soiling of nets, in the form of bioaccumulation can result in the attachment of shelled animals that could potentially damage the eyes of the fish.

This study provides a platform for research into growth strategies and the cause of size variation in Atlantic salmon in commercial conditions. Differences in growth rates in commercial conditions suggest that some individuals are not growing to their full potential. This potential may be realised by the development of production techniques that take different categories of fish into account. Within a sea cage, feed should be widely dispersed to decrease the possibility of monopolisation by dominant individuals. In addition, as small individuals were observed in the lower section of the experimental tanks (Chapter 3), the introduction of food at the middle or lower levels of cages may promote feeding in these individuals. In behavioural studies of small groups of salmonids, aggression was decreased or eliminated by providing shelters (McCarthy,

2000). Due to the logistical restrictions imposed by commercial conditions, shelters could not be provided in sea cages. However, an alternative, such as strips of black polythene or tightly woven netting hanging in the water column may provide shelter for subordinate fish.

Currently, very small fish are discarded during grading and routine husbandry, however it is clear that they do have the ability to grow (chapter 5). In order to assess the economic impact of discarding these individuals, accurate records should be kept on the numbers of fish removed during grading and the number of mortalities with no evidence of disease. This process would determine the usefulness of further research into encouraging these fish to grow.

If further investigation reveals that a number of small non-growing individuals in commercial conditions, ways of reducing the extent of these fish should be investigated. This is important from the aspects of welfare and overall production, but may not be beneficial economically. The described study demonstrated that non-growing fish grew following the summer solstice and harvesting may improve if this is taken into account. A slight delay in harvesting might result in the inclusion of small individuals that have recently rapidly increased in condition and weight. Alternatively, light manipulations may be used trigger an increase in growth of small fish in poor condition.

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APPENDIX I:

LATIN NAMES

Common name	Chapter
Crustacea	
aphids (*Pemphigus betae*)	3
butterfly (*Quadricalicarifera punctella*)	2
crab spiders (*Mesumenoides formosipes*)	6
prawns (*Macrobrachium rosenbergeii*)	5
Aves	
chickens (*gallus gallus)*	4, 5, 6, 7
greylag geese (*Anser anser*)	1
pigeons (*Columba livia)*	3
willow tits (*Parus montanus*)	1, 7
yellow-eyed juncos *(Junco phaeonotus)*	1
Pisces	
Salmonidae	
Artic charr (*Savelinus alpinus*)	2, 3, 4, 5, 6
Atlantic salmon (*Salmo salar)*	1 - 7
brown trout (*Salmo trutta*)	1, 2, 3, 5, 7,
coho salmon (*Oncorhynchus kisutch*)	2, 3, 6, 7
rainbow trout (*Oncorhynchus mykiss*)	1 - 7

Others (Pisces)

Atlantic halibut (*Hippoglossus hippoglossus*)	1, 2
common carp (*Cyprinus carpio*)	2, 5
coral reef damselfish (*Dascyllus albisella*)	1
green swordtail fish (*Xiphorus helleri*)	1
greenback flounder (*Rhombosolea tapirina)*	2, 3
medaka (*Oryzias latipes*)	1, 3, 6
midas cichilids (*Cichlasoma citronellum)*	2
pumkinseed sunfish (*Lepomis gibbosius)*	2
red sea bream (*Pagrus major*)	5
sturgeon (*Acipenser transmontanus*)	2, 5
tilapia (*Oreochromis niloctinus*)	1, 2
turbot (*Scophthalmus maximus*)	2
whitefish (Coregonus laveratus)	2

Mammalia

African dogs (*Lycaon pictus)*	1
baboons (*Papio sp*)	4
badgers (*Melus melus*)	3, 7
cats (*Felis catus*)	4
elephants (*Loxodonta africana*)	1
goats (*Oreamns americanns*)	1, 6
grasshopper mice (*Onychomys leucogaster*)	5
humans (*Homo Sapiens*)	4
pigs (*Sus scrofa*)	1, 2, 3
red deer (*Cervus elaphus*)	1, 7
seals (*Mirounga angustirostis*)	1
sheep (*Ovis aries)*	5
stumptail maque (*Alacaca arctoides*)	1